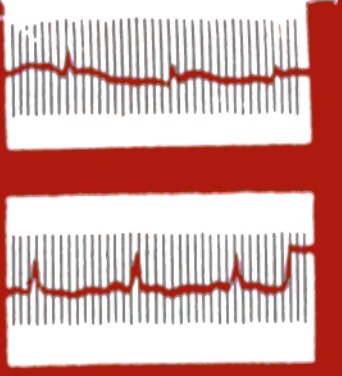
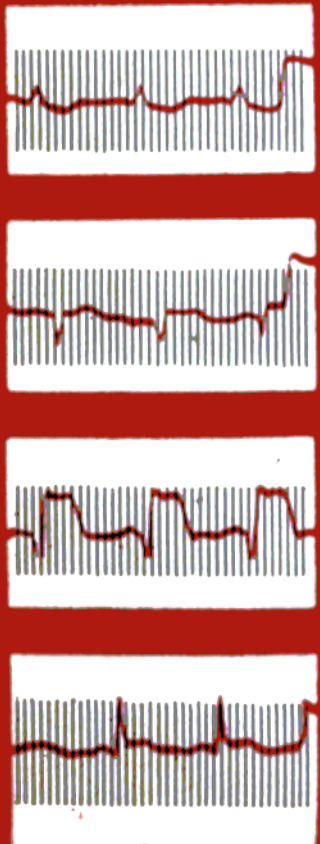


ATHEROSCLEROSIS AND THROMBOSIS



ATHEROSCLEROSIS AND THROMBOSIS

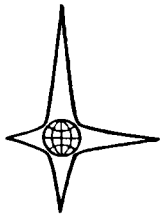
Prof. ALEXANDER MYASNIKOV, M. D., Fellow of the USSR Academy of Medical Sciences, was a prominent Soviet therapeutist, member of the Committee of the International Society of Internal Medicine and of a number of foreign cardiological societies, winner of the "Golden Stethoscope" international prize. He has published 120 scientific papers, 8 monographs and text-books of propedeutics for medical students.

Alexander Myasnikov's scientific studies are devoted to the basic problems of internal medicine—pathology of the liver, hypertension and atherosclerosis. He developed a new theory of hepatites with emphasis on two pathological forms—the epithelial and the mesenchymal.

His monograph on HYPERTENSIVE DISEASE presents an original view of the essence of hypertension, according to which the latter is due not only to disturbances of the cortical neurodynamics but to complex neurovisceral relationships as well.

Very important are his studies on atherosclerosis, which demonstrate the role of vitamins, hormones and neurotropic drugs in the development and prophylaxis of this disease.

Alexander Myasnikov has worked out methods for clinical diagnosis of atherosclerosis and drawn up the world's first classification of this disease taking into account its stage-by-stage progression. The all-important problem of relationships between atherosclerosis and hypertensive disease has been presented in a new light, and a conception has been advanced regarding the existence of a single disease manifested simultaneously or in succession by syndromes of atherosclerosis and hypertension.



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На английском языке

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AND
THROMBOSIS

EDITED

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FELLOW OF THE USSR ACADEMY
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THE USE OF LABELED ATOMS FOR INVESTIGATION OF CHANGES IN THE RATE OF SYNTHESIS AND DISINTEGRATION OF LIPOPROTEINS IN EXPERIMENTAL ATHEROSCLEROSIS

M. G. KRITSMAN, E. N. LEVITOVA

Although it is generally stated in the literature that certain classes of lipoproteins play a substantial role in the pathogenesis of atherosclerosis, the exchange between these complex lipid compounds and proteins, i.e., their synthesis and disintegration, has been little studied so far.

Analysis of the literature indicates that the overwhelming majority of studies on lipoproteins in atherosclerosis were limited mainly to determining changes in the correlations between α- and β-lipoproteins (M. V. Bavina, and M. Y. Melikova; M. G. Kritsman and M. V. Bavina; Gofman et al.; Pratt; Barr et al.; Bossak et al.; Rosenberg et al.).

As a result of this, there is today largely indirect evidence characterizing lipoprotein metabolism, from which it is difficult to infer a correct notion of the dynamics of these complex compounds in atherosclerosis.

Meanwhile, for a detailed knowledge of the mechanism of atherosclerosis pathogenesis and for planning a directed influence aimed at correcting the deranged lipoprotein metabolism which, in the view of certain authors, is responsible for specific structural changes in the vessel wall (Gerö et al.), it is necessary to discern clearly whether the disturbance of lipoprotein metabolism and excessive accumulation of lipoproteins occur in atherosclerosis as a result of intensified synthesis or delayed disintegration, as well as to find out in which degree each of the components of lipoproteins is responsible for a metabolic disturbance in the entire complex compound.

In ten Chinchilla rabbits weighing 3 kg each atherosclerosis was induced by the conventional method of N.N. Anichkov, i.e., by feeding the animals with cholesterol during 120 days. Small quantities of cholesterol (0.1 g per kg of body weight) were used in the experiments so as to avoid a high-degree hypercholesterolaemia. Ten normal rabbits of the same breed and weight were used for control. Investigation of the rate of synthesis and disintegration of lipoproteins and their protein component in experimental atherosclerosis was carried out with the aid of tracer elements.

At the end of cholesterol feeding, normal and atherosclerotic rabbits were given one intra-abdominal injection of sodium acetate labeled with C^{14} in the carboxyl group, in a dose of 0.3 mCu per kg of body weight. Blood samples were taken from rabbits of both groups at different time intervals (3, 5, 7, 24, 90 hours) after radioactive acetic acid injections. Blood serum lipoproteins were separated by electrophoresis into α and β fractions which were then determined quantitatively from their optic density and radioactivity. The intensity of the synthesis and disintegration of these compounds was expressed by the number of impulses.

Since it had been discovered in preliminary experiments that the radioactivity in lipoproteins attains a maximum in 3-5 hours after the injection of a labeled substance to animals, the radioactivity discovered in the blood serum lipoproteins of experimental and control animals at these intervals reflected the rate of synthesis and after five hours the rate of their disintegration.

In addition, the rate of the synthesis and disintegration of the blood serum globulin fraction was studied with the aid of a tracer carbon of acetic acid for judgement on the dynamics of the protein component of lipoproteins.

This method of investigation is rather extensively used for the study of the amino acids synthesis rate with their subsequent utilization by proteins, inasmuch as in the synthesis process the tracer carbon of acetic acid takes part in the formation of amino acids and, having become their component, may reflect their transformation into protein molecules, i.e., their synthesis. For examination of the rate of synthesis and disintegration of the above-mentioned protein fraction (protein component of lipoproteins) the blood serum proteins were broken up into fractions after Korner and Debro's method. The globulin fraction thus obtained was repeatedly washed with trichloroacetic acid, alcohol and ether and other organic solvents in order to remove lipids.

The proteins thus treated were then examined for radioactivity.

Fig. 1 gives average data for ten experiments collected in investigation of the rate of synthesis and disintegration of α-lipoproteins of the blood serum of control and atherosclerotic rabbits. Comparison of the degree of radioactivity found in the early periods of time (3-5 hours) characterizing, as it has been mentioned, the synthesis of the investigated lipoproteins, makes it clear that

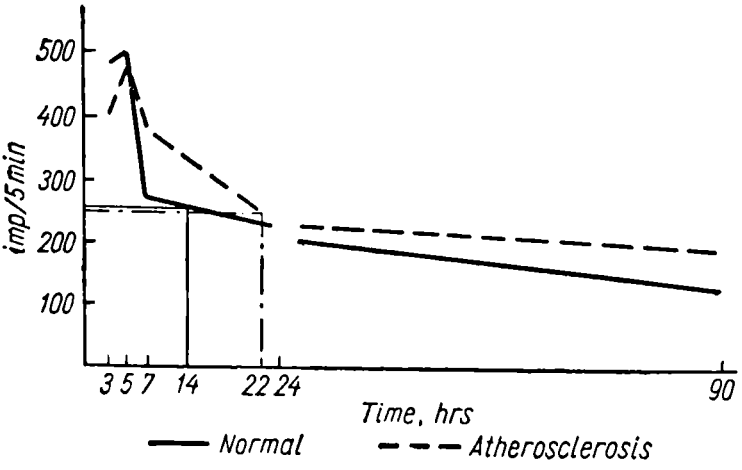

Fig. 1. Synthesis and disintegration of blood serum α-lipoproteins.

On axis of ordinate are indicated imp/5 min. On axis of abscissa is indicated time in hours. Continuous line indicates change in radioactivity of α-lipoproteins in time under normal conditions. Intermittent line shows change in radioactivity of α-lipoproteins in atherosclerosis. Rectangle formed by continuous lines shows normal period of α-lipoproteins half-life. Rectangle formed by dash-and-dotted lines indicates period of α-lipoproteins half-life in atherosclerosis

in atherosclerosis these degrees differ but little from those discovered under normal conditions.

However, in comparing radioactivity data for the descending part of the curve obtained at a later time of investigation (7, 24, 90 hours) it was found that the decrease of radioactivity to 50 per cent below the initial values occurred slower in the atherosclerotic rabbits than in the control ones.

It is seen in Fig. 1 that in the control rabbits a 50 per cent decrease of radioactivity took place in 14 hours whereas in the test rabbits such a reduction was noted in 22 hours.

The data adduced indicate that the rate of α-lipoproteins synthesis in atherosclerosis practically remains unchanged and that a certain lengthening of their half-life occurs simultaneously.

The β-lipoprotein metabolism was studied in an analogous manner. The findings are presented in Fig. 2.

Upon comparison of the degrees of radioactivity of β-lipoproteins of normal and atherosclerotic rabbits in the early periods of time (3-5 hours) it is clearly seen that the radioactivity of β-lipoproteins in experimental atherosclerosis is considerably above normal. This points to the activation of β-lipoproteins synthesis during this disease. Comparing the degrees of radioactivity determined in the subsequent periods of time (7, 24, 90 hours) characterizing the rate of disintegration of this lipoproteins fraction one may note a considerable deceleration of β-proteins disintegration in atherosclerosis.

This drawing shows that while in normal rabbits the 50 per cent reduction of radioactivity below the initial level occurred in 14 hours, in atherosclerosis such a decrease was noted only after 78 hours.

In this way, the data of investigations by means of tracer elements have indicated that the accumulation of β-lipoproteins in atherosclerosis is the result of their intensified synthesis and deceleration of disintegration, which serves to prolong their existence.

The amount of α- and β-lipoproteins in atherosclerotic and normal rabbits was determined, as it has been mentioned, by their optic density and radioactivity.

Fig. 3 shows the percentage of α- and β-lipoproteins in test and control rabbits and an adequate coincidence of the results obtained by the use of these two methods.

Fig. 4 presents data obtained by investigation of the rate of synthesis and disintegration of the blood serum globulin fraction produced in experiments with radioactive acetic acid. As it is seen, the radioactivity of this protein fraction with which lipids in the main combine to form lipoproteins practically does not differ from normal in atherosclerosis.

These data warrant the following conclusions:

(1) in experimental atherosclerosis the rate of synthesis and

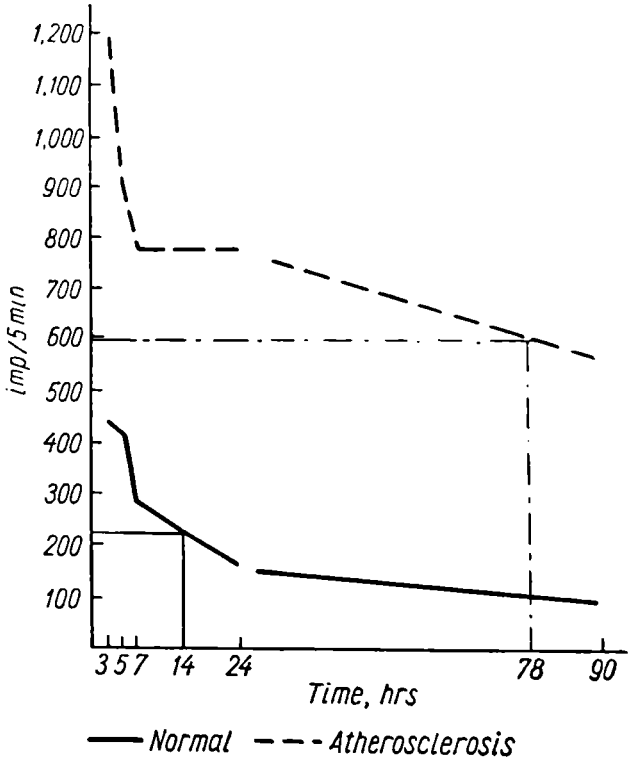

Fig. 2. Synthesis and disintegration of blood serum β-lipoproteins. Same designations as in Fig. 1

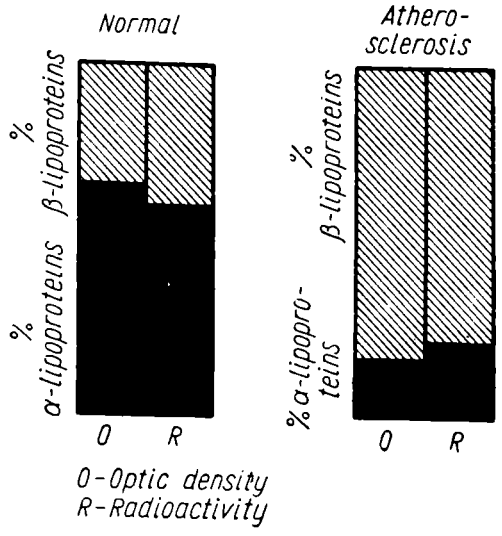

Fig. 3. Proportional relation of α- and β-lipoproteins.

Black columns show percentage of α-lipoproteins. Shaded columns indicate percentage of β-lipoproteins. The first two columns show normal proportion of β-lipoproteins. The other two columns show proportion of β-lipoproteins in atherosclerosis. *O*—optic density; *R*—radioactivity

disintegration of the protein component of lipoproteins remains unchanged;

(2) the ways of conversion of acetic acid into individual amino acids with their subsequent utilization in the formation of proteins are not disturbed in this disease either.

In addition to this, experiments were carried out in which radioactive proteins were subjected to complete acid hydrolysis with subsequent chromatography of amino acids and calculation of radioactivity. In these procedures several radioactivity peaks were obtained corresponding to individual amino acids.

The series of classical investigations carried out by Schöencheimer using the tracer element method proved that in a normal adult body all its component parts, including the proteins and lipids of organs and tissues, are constantly synthesized and disintegrating, and that the retention of the form and structure of a mature cell or tissue of the body is the result not of a static state of the body constituents but a dynamic state in which the processes of synthesis and disintegration occur with identical intensity. In pathology this correlation of oppositely directed processes may change.

The differences we have discovered in the rate of synthesis and disintegration of the lipid component of β-lipoproteins in atherosclerosis point to a disturbance in its circulation resulting

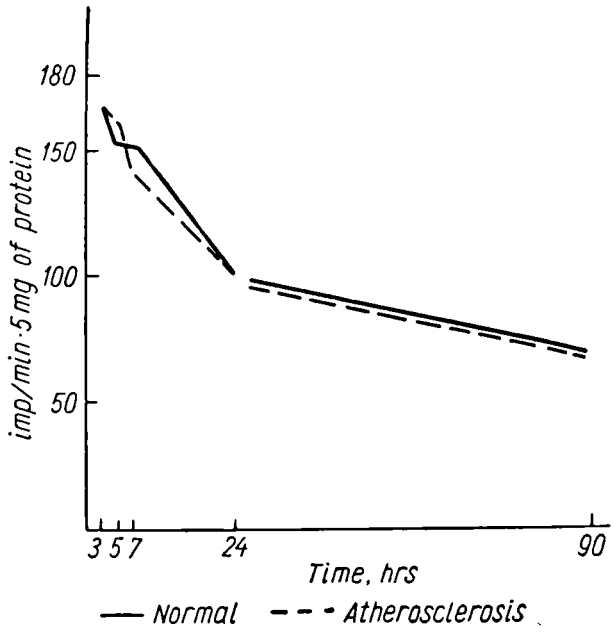

Fig. 4. Synthesis and disintegration of blood serum globulins.

On axis of ordinate are indicated imp/min 5 mg of protein. On axis of abscissa is indicated time in hours. Continuous line shows change in radioactivity of globulins under normal conditions. Dotted line indicates change in radioactivity of globulins in atherosclerosis

in a longer life of the lipid molecules which in its turn increases the probability of their interaction with protein and of excessive lipoprotein formation.

In view of this, measures for correcting a disturbed lipoprotein metabolism in atherosclerosis should be directed to the activation of the enzymatic systems catalysing the disintegration of the lipid predecessor of the lipoprotein complex.

CONCLUSIONS

1. The rate of synthesis and disintegration of lipoproteins was studied by means of tracer elements under normal conditions and in experimental atherosclerosis.

2. It has been established that in experimental atherosclerosis the rate of α-lipoprotein synthesis practically differs but little from normal.

3. It has been found that in experimental atherosclerosis the rate of α-lipoprotein disintegration is somewhat decreased. The average time of α-lipoprotein half-life under normal conditions is 14 hours, in atherosclerosis 22 hours.

4. It has been shown that the intensity of β-lipoprotein synthesis in experimental atherosclerosis is considerably higher than normal.

5. In experimental atherosclerosis the β-lipoprotein disintegration is considerably delayed as compared to normal. The normal half-life of β-lipoprotein is 14 hours, in atherosclerosis approximately 78 hours.

6. The changes discovered in the rate of synthesis and disintegration of lipoproteins are mainly caused by changes in the lipid part of these compounds.

7. The data obtained permit the conclusion that the development of experimental atherosclerosis is associated with considerable disturbances in the renewal of the lipid component of lipoproteins owing to their delayed circulation.

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INVESTIGATION OF GLUCOSO-6-PHOSPHATE DEHYDROGENASE ACTIVITY IN EXPERIMENTAL ATHEROSCLEROSIS

S. ALEKSEYEVA

During the past few years a vast amount of factual material has been accumulated on the distribution of enzymes in various organs and tissues. It has been found that while the concentration of individual enzymes varies with different tissues, the qualitative composition of enzymes in these tissues is identical, i.e., all types of metabolism are represented in different tissues, although in a variable degree.

The research into the enzymatic activity of aortic tissue in atherosclerosis was begun only in recent years, so only certain tentative data which have no systematic character are available on this problem.

For example, Zemplényi and Grafnetter, Loida and Zemplényi, Kittinger et al., Wegman and Fouquet, and a number of other investigators studied the activity of certain enzymes of the aorta in human atherosclerosis and the enzymes of the intima of a number of vessels in experimental animals with spontaneous vascular lesions. Their investigations have shown that the vascular system takes an active part in metabolism owing to the large amount of enzymes present in its composition, which are responsible for the principal reactions of the glycolytic cycle, the pentoso-phosphate way, the tricarboxylic acids cycle, phosphorus metabolism, and a number of others.

It is possible that the study of the metabolic processes occurring in the vessels will help understand the role of the vessel wall in the pathogenesis of atherosclerosis.

The present research continues the earlier investigations of the activity of certain enzymes of the tricarboxylic acids cycle.

It presents data on the activity of an enzyme in the pentoso-phosphate or apotomic way of transformation of carbohydrates—glucoso-6-phosphate dehydrogenase in the aorta, liver and erythrocytes of rabbits with experimental atherosclerosis (kept on cholesterol diet during 5, 20, 30, 40 and 50 days). The experiments were carried out on 30 test and 15 control male Chinchilla rabbits weighing from 2.5 to 3 kg.

Experimental atherosclerosis was induced by N.N. Anichkov's method in rabbits which were fed with a 20 per cent cholesterol solution in sunflower-seed oil in doses of 0.1 g of pure cholesterol per kg of body weight. The rabbits were slaughtered by decapitation, the aorta and liver were quickly extracted and thoroughly dried from blood on filter paper; all subsequent manipulations were performed in the cold.

The activity of glucoso-6-phosphate dehydrogenase in the aorta was studied in extracts from sections in the following way: discs of identical diameter (7 mm) were excised from strictly defined parts of the aorta and were used for the preparation of sections which were then subjected to extraction in tris buffer solution (pH 8.5) during ten minutes, whereupon the enzymatic activity was measured immediately.

For investigation of the activity of the above liver enzyme, a 2.5 per cent liver homogenate was prepared in a 0.25 M solution of saccharose. The liver homogenate was centrifugated at 3,500 rpm at zero temperature during 15 minutes. The supernatant liquid was taken for determination of the enzymatic activity.

The enzymatic activity in the erythrocytes was determined in erythrocyte hemolysates isolated from heparinized blood.

In our experiments we used a system which contained not only excessive substrate but also an excess of co-enzyme II (triphosphopyridine nucleotide). Under the influence of highly active nucleotidases, phosphatases and systems oxidating thiol compounds the co-enzymes of the tissues under investigation quickly disintegrate, as a result of which it is no longer the enzyme power that is the factor limiting the enzymatic reaction but the artificially produced co-enzyme deficit.

The activity of glucoso-6-phosphate dehydrogenase in all experiments was determined by the method of continuous spectrophotometry of the course of the enzymatic reaction at $\lambda = 340 m\mu$ within a period of 5 to 15 minutes (Glock and McLean).

The activity of glucoso-6-phosphate dehydrogenase in the aorta was calculated in micromoles of per-minute restored triphosphopyridine nucleotide per gram of soluble protein. The activity of this enzyme in the liver was expressed in units of optic density per mg of soluble protein of the supernatant liquid of the liver homogenate, and in the erythrocytes—in micromoles of per-minute restored triphosphopyridine nucleotide per 100 ml of erythrocytes.

To avoid errors connected with seasonal or alimentary factors, the investigations were in all cases carried out simultaneously in two experimental and one control animals.

Data on the activity of glucoso-6-phosphate dehydrogenase in the rabbit aorta at different periods of experimental atherosclerosis are presented in Table 1.

It was found during the experiments that a certain reduction in the activity of glucoso-6-phosphate dehydrogenase in the aorta

Table 1

ACTIVITY OF GLUCOSO-6-PHOSPHATE DEHYDROGENASE IN EXTRACTS FROM SECTIONS OF THE RABBIT AORTA (AVERAGE VALUES IN MICROMOLES OF PER-MINUTE RESTORED TRIPHOSPHOPYRIDINE NUCLEOTIDE PER GRAM OF SOLUBLE PROTEIN)

Experiment		Control
Term of experiment, days	Enzymatic activity	Enzymatic activity
5	19.7±0.7	21.0±1.2
20	17.2±1.8	21.3±1.1
30	21.1±1.6	21.0±0.4
40	18.2±0.8	22.5±1.3
50	17.5±1.5	22.1±0.5

takes place as early as five days after the cholesterol feeding of rabbits is started.

A somewhat larger reduction of the activity of glucoso-6-phosphate dehydrogenase in the aorta was noted during subsequent cholesterol feeding of rabbits. For example, on the 20th day of the experiment the enzymatic activity in the aorta was found to be equal to 17.2 U on the average as compared to 21.3 U in control.

In the next series of experiments (30 days) the average values of enzymatic activity in experiments and in control were equal, which was probably due to the mobilization of some protective mechanisms during the experimental period.

With a longer term of experiment (40 days) there was again a decrease in the activity of glucoso-6-phosphate dehydrogenase in the aorta (to 18.2 U) as compared to 22.5 U in control.

Simultaneously, studies of the activity of glucoso-6-phosphate dehydrogenase in soluble proteins of the liver homogenate were carried out in the same group of experimental animals. The findings are presented in Table 2 which shows that the activity of glucoso-6-phosphate dehydrogenase was slightly reduced.

Comparison of data for each experimental and control animal in the sequence in which these values were obtained shows a reduction in the activity of this enzyme in the soluble liver proteins.

To ascertain the activity of glucoso-6-phosphate dehydrogenase in the intravital period, we undertook studies of the activity of this enzyme in erythrocytes of the rabbit blood at different stages of experimental atherosclerosis. This failed to reveal

Table 2

ACTIVITY OF GLUCOSO-6-PHOSPHATE DEHYDROGENASE IN SOLUBLE PROTEINS OF LIVER HOMOGENATE (AVERAGE VALUES IN UNITS OF OPTIC DENSITY PER MG OF SOLUBLE PROTEIN OF LIVER HOMOGENATE SUPERNATANT LIQUID)

Experiment		Control
Term of experiment, days	Enzymatic activity	Enzymatic activity
5	11.0±0.1	11.0±0.2
20	11.9±0.4	13.2±1.2
30	11.2±0.5	12.0±0.02
40	11.0±0.3	12.6±0.4
50	10.4±0.3	11.2±0.7

a substantial difference between the groups of experimental and control animals.

Thus, in experimental atherosclerosis the activity of glucoso-6-phosphate dehydrogenase in the aortic tissue reduces, the enzymatic activity in the liver tissue showing a certain tendency to reduction and remaining practically unchanged in the erythrocytes.

It is doubtless that the experiments made with intact aortic tissue give only an average value for all the cellular constituents present within it, and that a more detailed picture of enzymatic activity can be obtained by fractional centrifugation. However, the fairly regular reduction of glucoso-6-phosphate dehydrogenase activity in rabbits in experiments lasting for 20, 40 and 50 days shows that the processes of inhibition of enzymatic activity prevail during these periods.

It is evident that the enzymatic reactions ensuring normal conversion of carbohydrates in the aorta are partly blocked in the phase of oxidation of glucoso-6-phosphate and its transition to 6-phosphogluconate.

One of the possible causes of reduction in the activity of glucoso-6-phosphate dehydrogenase may be a disturbed protein-enzyme balance. The experiments of M. G. Kritsman and M. V. Bavina revealed a reduction of protein synthesis in experimental atherosclerosis. Another cause of reduction in glucoso-6-phosphate dehydrogenase activity may be a triphosphopyridine nucleotide deficit.

After a number of experiments it is well known at present that synthesis of fatty acids and cholesterol requires for its accom-

plishment the presence of triphosphopyridine nucleotide and that experimental atherosclerosis is attended with a sharp inhibition of cholesterol synthesis in various organs and tissues (M. G. Kritsman and M. V. Bavina; Frantz et al.).

Data on the immediate determination of the level of triphosphopyridine nucleotide or its structural element, nicotinic acid, are at the present time contradictory.

An indirect evidence of pyridine nucleotide deficit may be found in data furnished by Kirk et al. to the effect that during atherosclerosis in man the coronary arteries show a decrease in the enzymatic activity of lactic dehydrogenase whose catalytic activity may be contingent on pyridine nucleotide synthesis.

Of course, changes in the activity of enzymes may have a chain character, which makes it difficult to choose the main link in a disturbed enzymatic catalysis of substance conversion.

At present it is difficult to decide whether the reduction in glucoso-6-phosphate dehydrogenase activity is a compensatory reaction of the body or the result of an injury. Further experimental research will give the answer to this problem.

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CHANGES IN THE AORTIC AND LIVER PROTEINS DURING THE INCIPIENT PERIOD OF EXPERIMENTAL ATHEROSCLEROSIS

V. F. ZAITSEV

In addition to a disturbed lipid metabolism, an important factor in the pathogenesis of atherosclerosis is a disorder in protein metabolism (N. N. Anichkov and V. D. Tzinzerling; A. L. Myasnikov). In the blood serum of patients with atherosclerosis the albumin level is reduced and the level of β-globulins (V. M. Zaitsev), β- and γ-globulins (P. E. Lukomsky) and sometimes also of α-globulins (A. G. Teregulov and V. F. Bogoyavlensky; Houss and Leist, Pagé and Lewis) is increased. In experimental atherosclerosis the serum also has a reduced albumin content and an increased level of β-globulins (Fishberg et al.), β- and γ-globulins (M. V. Bavina and M. G. Kritsman; T. N. Lovyagina).

Various changes in proteins are noted during atherosclerosis in the arterial wall. With the progression of experimental atherosclerosis, protein synthesis in the aorta at first increases and at later stages decreases, while its disintegration becomes more intensive (M. G. Kritsman and M. V. Bavina). The proteins of the arterial wall are composed of proteins soluble in water and saline dissolvents (albumins, globulins) and insoluble (collagen, elastin) (Balo et al.). In atherosclerosis the elastin level in the aorta considerably decreases and the collagen level increases somewhat (Buddecke). The collagen level increases already in the early stages of atherosclerosis (Noble et al.).

The liver in experimental atherosclerosis just as the aorta shows a decrease in the synthesis and an increase in the disintegration of protein (M. G. Kritsman and M. V. Bavina). Changes in the liver proteins affect the protein composition of the blood

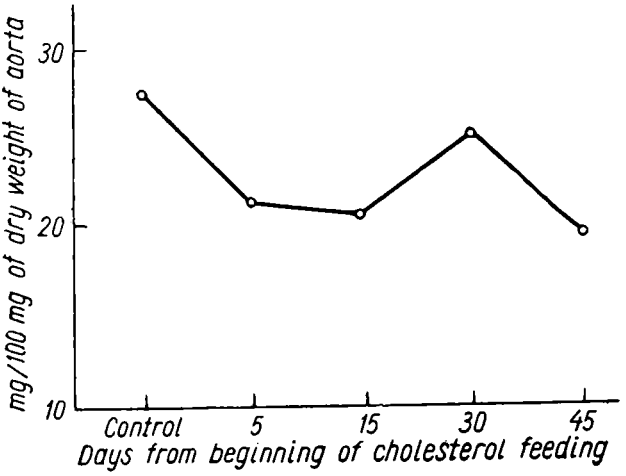

Fig. 1. Change in the level of soluble protein in the aorta in rabbits kept on a cholesterol diet during 5, 15, 30 and 45 days, in milligrams per 100 mg of dry weight

serum since it is in the liver that serum albumins and partly globulins are synthesized (S. Y. Kaplansky et al.; Miller; Peters and Anfinsen; Roberts and White; Sorof and Cohen). It should also be noted that most of the liver proteins enter into the composition of enzymes (Fischer), including those catalysing protein and lipid metabolism. Therefore, a change in the protein composition of the liver cannot but affect the metabolism of the whole body.

All these data evidence that changes in the protein composition of the aorta and liver substantially contribute to the pathogenesis of atherosclerosis.

The subject of the present research was a study of changes in soluble proteins of the aorta and liver during the incipient period of experimental atherosclerosis prior to the appearance in the aorta of the first macroscopic signs of lipoidosis.

Observations were carried out on 50 Chinchilla rabbits weighing from 2.5 to 3 kg. The control group consisted of ten rabbits which were not given additional cholesterol. The remaining forty rabbits were daily given food with cholesterol dissolved in sunflower-seed oil in doses of 0.2 g per kg of body weight during 5, 15, 30 and 45 days. Each of these groups included ten animals. At the beginning and in the end of the experiment the amount of protein, protein fractions and cholesterol were determined in the blood serum. The investigations were carried out on a whole aorta from which the adventitia had been removed. The liver was drained of blood by means of perfusion with physiological saline. The aorta and a portion of the liver were frozen at 30 °C below zero and dried to a constant dry weight in vacuum above alkali. The soluble protein of the aorta and liver was extracted with a phosphate buffer at pH 7.4 in the following way. The aorta and a portion of the liver were minced and frozen with a phosphate buffer at 30 °C below zero, and then were thoroughly ground in a mortar at room temperature. This procedure was repeated five or six times after which the homogenate was centrifugated with cooling during 30 minutes at 3,500 rpm. The sediment was supplemented with an approximately equal amount of phosphate buffer, and the resulting suspension was kept in a refrigerator at a temper-

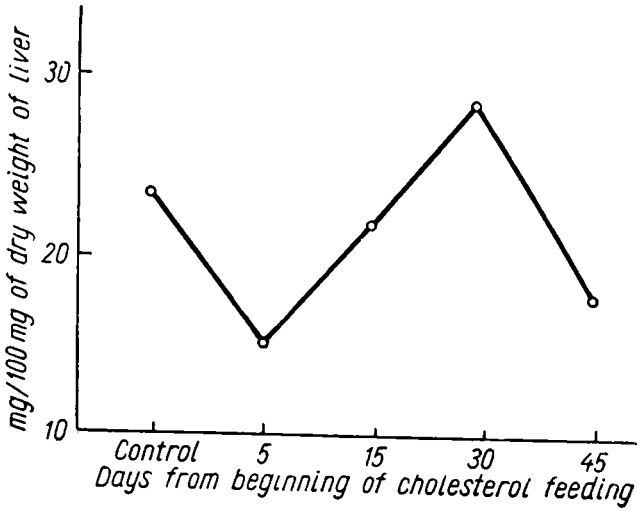

Fig. 2. Change in the level of soluble protein of the liver in rabbits kept on a cholesterol diet during 5, 15, 30 and 45 days, in milligrams per 100 mg of dry weight

ature of 2-4°C above zero during 24 hours and was then again centrifugated with cooling. The sediment was once again allowed to stand with phosphate buffer at a temperature of 2-4°C above zero during 3-4 hours. The supernatant liquid obtained after three consecutive extractions of soluble aortic and liver protein was collected and treated with ether. In this way a yellowish opalescent solution was produced in which the protein level was determined after Lowry. Paper electrophoresis of the liver and serum proteins was performed in a veronal-medinal buffer at pH 8.9 during 16-18 hours at 220 V. Electrophoresis of the aortic proteins was not performed because of the low protein concentration in the solution. The electrophoregrams were stained with amido black green 10 B, then cut into separate fractions and eluted with a 0.1 N NaOH solution in 50 per cent methanol, which was followed by colorimetry with a red filter. The total amount of protein extracted from the aorta and liver was calculated for 100 mg of dry weight of the organ. The fractions of the soluble liver protein were expressed in absolute values in view of the fact that the total amount of protein during cholesterol feeding changed considerably.

The change in the level of the soluble aortic protein is presented in Fig. 1. In the aorta of control animals the amount of soluble protein was on the average 26.8±3.4 mg per 100 mg of dry weight. In rabbits given cholesterol during 5 and 15 days, the level of soluble protein in the aorta was less than in control animals—20.8±1 and 19.9±1.9 mg per 100 mg on the average. In animals to which cholesterol was given during 30 days the soluble protein in the aorta was on the average 24.4±2.3 mg per 100 mg, i.e., its amount was larger than in the rabbits given cholesterol during 5 and 15 days. On the 45th day of the experiment the level of soluble protein in the aorta was low again—19.2±1.9 mg per 100 mg on the average.

In the liver of control animals the amount of soluble protein was 23.2±0.8 mg per 100 mg on the average. In rabbits given cholesterol during 5 days the level of soluble protein in the liver

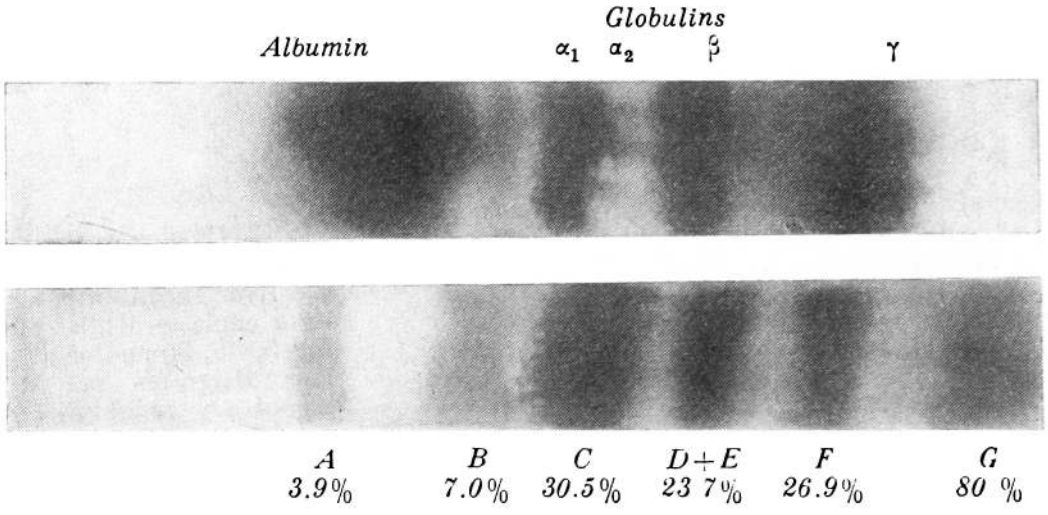

Fig. 3. Electrophoregrams of serum protein and soluble protein of the liver. The values of fractions are the average for 10 healthy rabbits in percentage to the total amount of protein

was less than in the control animals—15.1±0.7 mg per 100 mg on the average (Fig. 2). Rabbits examined 15 and 30 days after the beginning of cholesterol feeding were found to have more soluble protein in the liver than those which had been given cholesterol only for 5 days. Thus, on the 15th day of the experiment, the level of soluble protein in the liver was on the average 21.5±1.5 mg per 100 mg, and on the 30th day—27.8±2.5 mg per 100 mg on the average. In animals given cholesterol during 45 days the level of soluble protein in the liver was again, just as on the 5th day of the experiment, low—17.4±1.4 mg per 100 mg on the average.

Electrophoretic investigation of the fractions of soluble liver protein yielded additional data on its changes in animals kept on a cholesterol diet. The electrophoregrams of soluble liver protein in our experiments clearly showed six fractions, which are designated by alphabetical letters. The disposition of the soluble liver protein fractions with regard to the protein fractions of the serum is presented in Fig. 3. Fraction A of the soluble liver protein was located in front of the serum albumins, fraction B in front of the α-globulins, fraction C between the α- and β-globulins, fraction D+E in front of the β-globulins, fraction F in front of the γ-globulins of the serum. The electrophoretic motility of fraction G was less than that of the serum γ-globulins. The amount of separate fractions of soluble liver protein in normal animals is shown in Fig. 3 (in this case in proportion to the total amount of soluble protein). The largest amount of protein

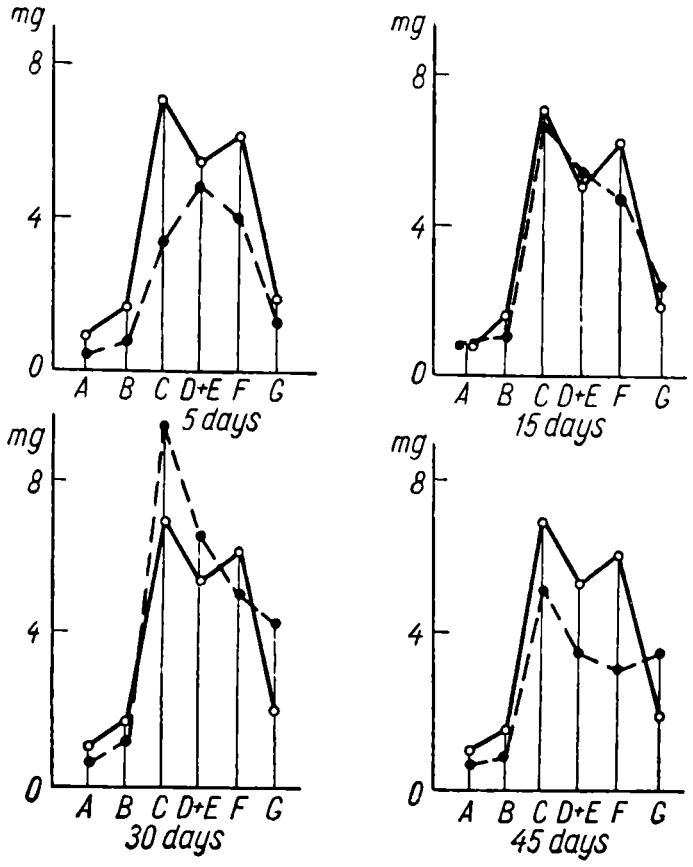

Fig. 4. Changes of soluble liver protein fractions in rabbits kept on a cholesterol diet during 5, 15, 30 and 45 days. The fractions are given in absolute values. White circles and continuous lines indicate protein fractions in healthy rabbits; shaded circles and dashed lines indicate fractions in rabbits kept on a cholesterol diet

was contained in fractions C, D+E and F. The amount of protein in fractions A, B, and G was relatively small.

Changes in the fractions of soluble liver protein in animals kept on a cholesterol diet are presented in Fig. 4. The average numerical indices are given in Table 1. Rabbits given cholesterol during 5 days were noted to have a smaller amount of all fractions of soluble protein than the control animals. In this way, the reduction in the total amount of soluble protein in the liver in this period was caused by a decrease in the amount of all its fractions. In rabbits examined 15 days after the beginning of cholesterol feeding the level of almost all fractions of soluble liver protein was approaching normal. Only the amount of fraction F remained lower than in the control animals, but nevertheless it was larger than in the rabbits given cholesterol food for 5 days. Thirty days after the beginning of the experiment the protein content of frac-

Table 1

AMOUNT OF SOLUBLE AORTIC AND LIVER PROTEIN AND LIVER PROTEIN FRACTIONS LEVEL IN CONTROL AND CHOLESTEROL-FED RABBITS (AVERAGE VALUES IN MG PER 100 MG OF DRY LIVER WEIGHT)

Number of animals	Term of cholesterol feeding	Soluble aortic protein	Soluble liver protein	Fractions of soluble liver protein					
				A	B	C	D+E	F	G
10	Control	26.8±3.4	23.2±0.8	0.89±0.06	1.65±0.2	7.06±0.4	5.52±0.2	6.23±0.4	1.89±0.1
10	5 days	20.8±1.0	15.1±0.7	0.47±0.03	0.86±0.08	3.48±0.6	4.91±0.3	3.97±0.5	1.45±0.3
10	15 days	19.9±1.9	21.5±1.5	1.4 ±0.4	1.23±0.2	6.60±0.6	5.58±0.6	4.67±0.5	2.42±0.3
10	30 days	24.4±2.3	27.8±2.5	0.83±0.1	1.41±0.3	9.51±0.8	6.60±0.7	5.10±1.1	4.35±0.7
10	45 days	19.2±1.9	17.4±1.4	0.87±0.1	0.9 ±0.1	5.36±0.1	3.65±0.05	3.07±1.1	3.46±0.4

tions C, D+E and G was found to be considerably above normal. On the 45th day of the experiment, just as on the 30th day, fraction G remained at a high level. The level of all the other soluble protein fractions in this period, however, was lower than in the control rabbits. As is seen in Fig. 4, on the 30th and 45th day of the experiment the correlation between the fractions of soluble liver protein was changed considerably.

In the serum the amount of total protein did not show any substantial changes and was equal to 7.7-8.6 g per cent on the average. In investigation of the serum protein fractions one could note a gradual decrease in the level of albumins and an increase in that of β-globulins, beginning from the 15th day of cholesterol feeding (Table 2). Changes in the other serum protein fractions were insignificant.

The degree of cholesterolaemia increased depending on the duration of cholesterol feeding of the animals. The serum of control animals contained 80±6.2 mg per cent of cholesterol on the average; the figures for rabbits given cholesterol for 5 days were 188±24.7 mg per cent; for 15 days 690±61.2 mg per cent; for 30 days 945±150.3 mg per cent; for 45 days 1,285±143.1 mg per cent on the average.

The aortas of control rabbits and animals given cholesterol during 5, 15 and 30 days showed no macroscopic signs of lipoidosis. Only the rabbits kept on a cholesterol diet during 45 days had small lipoid patches in the aorta, mainly in its arch and of the junctions with larger vessels.

DISCUSSION

Protein changes in the aorta and liver of rabbits took place as early as on the 5th day of cholesterol feeding. In this early period, both the aorta and the liver showed a decrease in the level of soluble protein. In the liver this decrease was more significant (35.4 per cent on the average) than in the aorta (22.4 per cent on the average). Later, however, on the 15th day of cholesterol feeding the soluble protein level in the liver seemed to restore after the preceding early decrease. The correlation between the fractions of soluble liver protein in this period also approached normal. Subsequently, on the 30th day of the experiment the soluble protein level in the liver showed a still larger increase and even rose slightly above normal. This increase took place at the expense of a part of the soluble liver protein fractions. In this period restoration of the amount of soluble protein was observed in the aorta also. Finally, on the 45th day of the experiment the liver and aorta also showed another decrease in the soluble protein content, which coincided with the appearance of macro-

Table 2

TOTAL PROTEIN AND SERUM PROTEIN FRACTIONS IN NORMAL ANIMALS AND IN CHOLESTEROL-FED RABBITS

Number of animals	Term of cholesterol feeding	Total serum protein, g %	Serum protein fractions, %				A/D index
			albumins	globulins			
				α	β	γ	
10	Control	7.7±0.4	62.6±1.6	11.5±1.4	12.1±1.2	13.8±0.9	1.7±0.1
10	5 days	8.6±0.2	63.5±2.5	13.0±1.5	11.9±0.6	11.6±0.9	1.7±0.2
10	15 days	8.6±0.4	58.2±2.0	12.0±1.4	13.6±0.8	12.6±0.9	1.6±0.1
10	30 days	8.5±0.4	60.8±3.3	10.4±1.3	15.2±1.0	13.6±2.5	1.5±0.2
10	45 days	7.8±0.4	60.6±2.1	10.8±0.7	14.5±1.1	14.1±1.5	1.5±0.1

scopic signs of aortic lipoidosis. In the serum no such undulatory changes in proteins took place. One could note only a gradual reduction in the amount of serum albumin and an increase of the β-globulin level.

In the protein changes observed in the incipient period of atherosclerosis (lipoidosis) special note should be taken of the period when in the liver and then in the aorta the soluble protein level following an initial decrease became restored and approached normal. It may be presumed that this resulted from the activation of protective reactions of the body in response to disorders caused by introduction of a large amount of cholesterol. These protective reactions which manifested themselves by a restoration of the soluble protein level had formerly been observed in the liver and later in the aorta. Possibly the restoration of the protein content of the aorta was the result of activation of protective reactions in the liver, an organ having a variety of functions with regard to protein metabolism. This can be supposed from comparison of the terms of restoration of the protein structure in these organs as well as from the fact that the increase in the protein level in the liver in this period was considerably larger than in the aorta. Later, the protective reactions seemed to be exhausted which in our investigations was manifested by another decrease in the soluble protein level in the liver and the aorta. This coincided with the appearance of macroscopic signs of aortic lipoidosis.

Thus, during the origination of experimental atherosclerosis and prior to the appearance of macroscopic signs of aortic lipoidosis protein changes are observed in the liver and aorta; at first the level of soluble proteins declines, then the protective reactions seem to be brought into play, and the amount of proteins in these organs is restored, and, finally, there follows a period of another decrease of the protein level in the liver and the aorta. In the latter period the aorta shows signs of lipoidosis.

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THE INFLUENCE OF THYROIDIN ON SOLUBLE PROTEINS OF THE AORTA, LIVER AND BLOOD SERUM IN EXPERIMENTAL ATHEROSCLEROSIS

L. A. MYASNIKOV

The hormones of the thyroid gland play an important part in the pathogenesis of atherosclerosis. There is a clear understanding of their influence on the cholesterol content of the blood. It is believed that the hypocholesterolaemic effect of the thyroid hormones is responsible for the delay in the development of atherosclerosis (T. A. Sinitsina; V. V. Tatarsky and V. D. Tzinzerling).

In our previous papers we discussed the action of the thyroid hormones on cholesterol metabolism. In particular, a study was made on the influence exerted by thyroidin on the distribution of labeled cholesterol in the organs of rabbits with experimental atherosclerosis, as well as a change in the total cholesterol level in various tissues under the influence of thyroidin (L. A. Myasnikov and V. F. Zaitsev).

The present paper describes the findings made in the study of the influence of thyroidin on protein metabolism, since changes in the latter during atherosclerosis are thought to be of substantial significance (N. N. Anichkov, V. D. Tzinzerling, A. L. Myasnikov).

The experiments were staged on 32 rabbits weighing from 2 to 2.5 kg. To study the action of thyroidin in normal animals, 16 rabbits were kept on the conventional diet, 8 of them being given thyroidin in doses of 0.06 g/kg during 45 days. To ascertain the influence of thyroidin during alimentary atherosclerosis, another group of 16 rabbits were fed with cholesterol in doses of 0.2 g/kg during 45 days; 8 of them were in addition to cholesterol given thyroidin in doses of 0.06 g/kg during the same period.

The protein content of the blood serum and the protein fractions were determined in all animals by Lowry's method and paper electrophoresis

AVERAGE LEVEL OF SOLUBLE PROTEIN IN TISSUES OF RABBITS

Groups of rabbits	Aorta	Liver			
	Protein, mg/100 mg	Protein mg/100 mg	Fractions of soluble		
			A	B	C
Healthy	20.5±2.3	23.2±0.8	0.9±0.06	1.6±0.18	7.0±0.4
Thyroidin-fed	18.2±1.9	27.2±3.1	1.3±0.07	1.6±0.2	9.7±0.4
Cholesterol-fed	19.2±1.9	17.4±1.4	0.9±0.1	0.9±0.6	5.4±0.1
Fed with cholesterol and thyroidin	18.1±1.8	21.1±2.2	1.0±0.1	0.9±0.1	6.3±0.2

respectively. The amount of soluble protein in the aorta and liver, as well as the protein fractions of the soluble liver protein was determined at the end of the experiment. For this purpose, the organs were drained of blood by means of perfusion with physiological saline. The aortas were stripped of the adventitia. The whole aorta was taken for examination and a part of the liver, always from one and the same lobe. The preparations were refrigerated at 30°C below zero, and then were reduced to a constant dry weight above alkali in a vacuum. After this, they were homogenized with phosphate buffer at pH 7.4 with multiple refrigeration at 30°C below zero. The soluble protein was extracted three times with the same buffer and treated with ether. The protein amount was determined by Lowry's method and re-calculated per 100 mg of dry organ weight. The protein fractions of the liver were investigated by means of paper electrophoresis in a veronal-medinal buffer at pH 8.9 followed by staining with amido black green 10 B, elution and colorimetry. The liver proteins were usually separated into six fractions. In view of the considerable variations in the total protein content of the liver, determination of the percentage of fractions was followed by their re-calculation into absolute figures which expressed them, just as the protein content, in milligrams per 100 mg of dry liver weight.

The influence of thyroidin on protein metabolism in normal rabbits was estimated by comparing the protein content of the aorta, liver and blood serum in 8 rabbits given thyroidin and kept on the conventional diet with the corresponding data for 8 normal rabbits.

The comparison of the results shows (see table) that in thyroidin-fed animals the average amount of soluble protein in the aorta was less (18.2±1.9 mg per 100 mg of dry weight) than in normal animals (20.5±2.3 mg per 100 mg); and in the liver it was larger

FERENT GROUPS

otein, mg/100 mg			Blood serum				
			Protein g/%	Protein fractions, %			
				albumins	Globulins		
)+E	F	G			α	β	γ
±0.2	6.2±0.4	1.9±0.15	7.7±0.4	62±1.6	12±1.4	12±1.2	14±0.9
±0.3	4.6±0.3	3.1±0.2	7.4±0.3	66±1.7	12±1.6	13±1.4	9±0.7
±0.05	3.0±1.1	3.5±0.4	7.8±0.4	60±2.1	11±0.7	15±1.0	14±1.5
±0.1	4.0±0.9	3.9±0.5	7.1±0.3	61±2.3	12±0.8	13±1.0	14±1.6

(27.2±3.1 and 23.2±0.8 mg per 100 mg respectively). The average protein content of the blood serum was almost identical in animals of both groups (7.4±0.3 g in thyroidin-fed and 7.7±0.4 g per cent in normal animals). Thus, the amount of protein in the aorta of thyroidin-fed rabbits was 88 per cent, in the liver 120 per cent and in the blood serum 96 per cent of the protein level in normal rabbits (Fig. 1).

An analysis of the protein fractions (Fig. 2) showed that the larger amount of protein in the liver of thyroidin-fed animals was in correspondence with a higher content of fractions A as compared to that in normal animals (1.3±0.07 and 0.9±0.06 mg), as well as fractions C (9.7±0.4 and 7.0±0.4 mg), D±E (6.9±0.3 and 5.5±0.2 mg) and G (3.1±0.2 and 1.9±0.15 mg). The liver fraction B was identical in animals of both groups (1.6±0.2 and 1.6±0.18 mg), and fraction F in thyroidin-fed rabbits was less than in normal animals (4.6±0.3 and 6.2±0.4 mg).

The protein fractions of the blood serum (Fig. 2) in thyroidin-fed rabbits contained more albumins (66±1.7 per cent) and less γ-globulins (9±0.7 per cent), than it was the case in normal rabbits (62±1.6 per cent and 14±0.9 per cent respectively). The average level of α- and β-globulins was identical in both groups (12±1.6 per cent and 12±1.4 per cent of α-globulins and 13±1.4 per cent and 12±1.2 per cent of β-globulins). It should be noted that the increase in the blood serum albumins content under the influence of thyroidin was combined with an increase

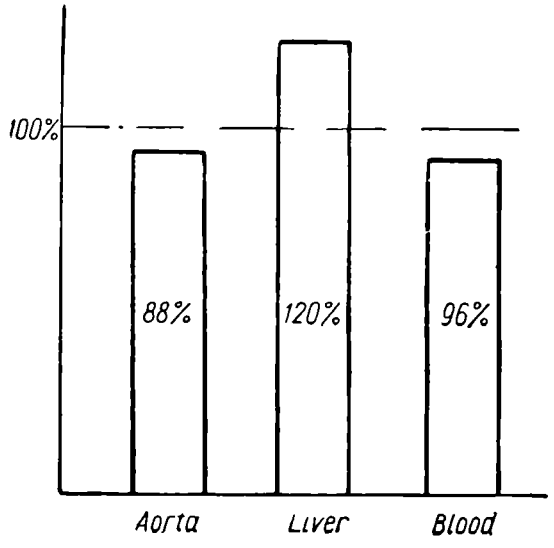

Fig. 1. The influence of thyroidin on the level of soluble protein in rabbit tissues.

The protein level in the tissues and blood of healthy rabbits is taken for 100 per cent. Columns indicate the protein content of the tissues and blood in thyroidin-fed rabbits in percentage to that in healthy animals

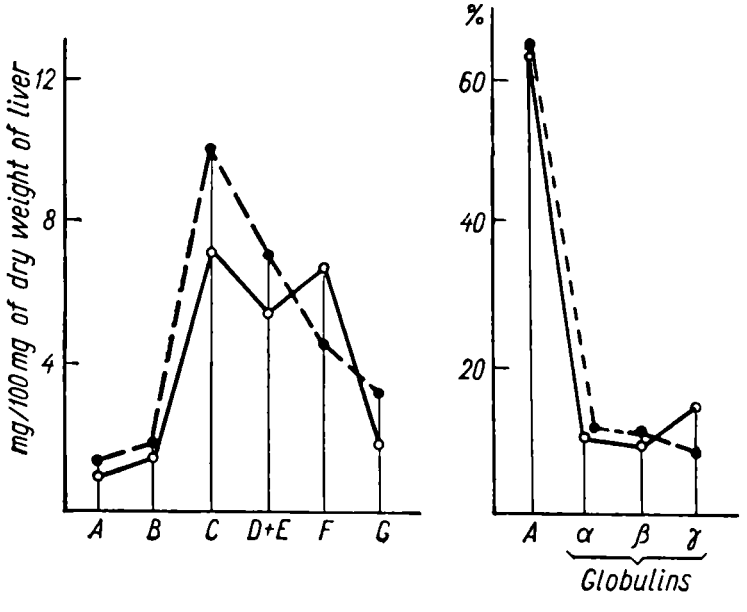

Fig. 2. Protein fractions of the liver (left) and blood serum (right) in healthy rabbits (continuous line) and in thyroidin-fed rabbits (intermittent line)

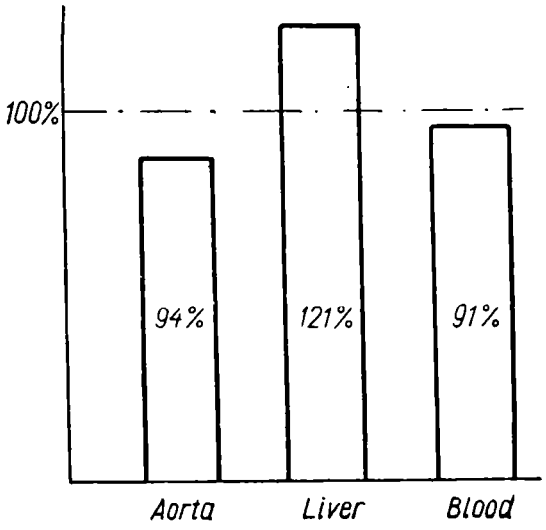

Fig. 3. The influence of thyroidin on the level of soluble protein in the tissues of rabbits with experimental atherosclerosis.

Protein level in the tissues and blood of animals with experimental atherosclerosis is taken for 100 per cent. The columns show the protein level in the tissues and blood of animals kept on a cholesterol-thyroidin diet in percentage to that in rabbits with experimental atherosclerosis

in the liver fraction A which is usually identified with serum albumin (S. Y. Kaplansky et al.).

The action of thyroidin on the soluble protein level in the aorta, liver and blood in animals with experimental atherosclerosis was in many respects similar to that in normal animals. As compared to the group of animals kept on a cholesterol diet, in rabbits fed with thyroidin and cholesterol simultaneously (see table) the average protein level in the aorta (18.1±1.8 mg) and in the blood serum (7.1±0.3 g per cent) proved somewhat lower and in the liver somewhat higher (21.1±2.2 mg). If the average protein level in rabbits fed only with cholesterol (19.2±±1.9 mg in the aorta, 17.4±1.4 mg in the liver and 7.8±0.4 g per cent in the blood serum) is taken for 100 per cent (Fig. 3), the average protein level in rabbits fed with thyroidin and cholesterol simultaneously will be 94 per cent in the aorta, 121 per cent in the liver and 91 per cent in the blood.

It should be noted that the amount of protein in the liver of animals kept on a cholesterol diet without thyroidin was considerably less than in normal rabbits. In animals fed with cholesterol and thyroidin simultaneously the amount of soluble protein in the liver was also less than in normal animals, yet larger than in those fed with cholesterol alone (Fig. 4). It can be presumed, therefore, that during the development of experimental atherosclerosis the amount of protein in the liver tends to decrease, and administration of thyroidin simultaneously with cholesterol inhibits this decrease.

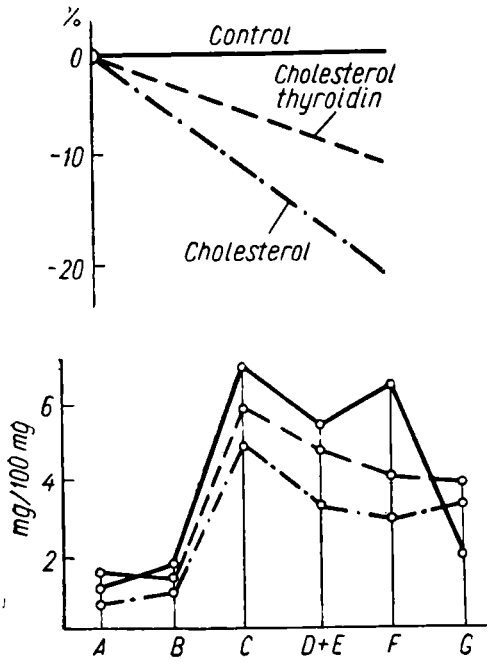

Fig. 4. Change in soluble liver protein (above) and liver protein fractions (below) in rabbits with experimental atherosclerosis under the influence of thyroidin. Continuous line indicates healthy animals, intermittent dotted line those kept on a cholesterol diet, and intermittent line those kept on a cholesterol-thyroidin diet

The average level of most of the liver fractions in animals with alimentary atherosclerosis was reduced as compared to normal animals (A—0.9±0.1 mg; B—0.9±0.16 mg; C—5.4±0.1 mg; D+E—3.7±0.05 mg; F—3.0±1.1 mg; G—3.5±0.4 mg). In animals fed with cholesterol and thyroidin simultaneously the level of the liver fractions (A—1.0±0.1 mg; B—0.9±0.1 mg; C—6.3±0.2 mg; D+E—5.0±0.1 mg; F—4.0±0.9 mg; G—3.9±±0.5 mg) differed less from the level of protein fractions in the liver of healthy animals than was the case with rabbits kept on a thyroidin-free cholesterol diet.

The protein fractions of the blood serum in animals with experimental atherosclerosis and in those fed with thyroidin and cholesterol simultaneously did not differ from each other significantly, except for the β-globulins fraction (see table); the latter increased in experimental atherosclerosis whereas administration of thyroidin simultaneously with cholesterol prevented this increase.

The role of changes in protein metabolism in atherosclerosis derives if only from the fact that the lipids pathogenetically associated with atherosclerosis form complexes with proteins. Indeed, the blood of patients with atherosclerosis is usually found to have a decreased albumin content and an increased level of β- and α- or β- and γ-globulins of the blood serum also during induction of experimental atherosclerosis (M. V. Bavina,

M. G. Kritsman, V. M. Zaitsev, P. E. Lukomsky). A study of the processes of protein synthesis and disintegration in various organs in experimental atherosclerosis has shown that in the early stages of atherosclerosis there is a certain intensification of protein synthesis in various tissues (including liver tissue) and its reduction in the later stages (M. G. Kritsman, M. V. Bavina). Protein disintegration, particularly in the aorta, in experimental atherosclerosis proves to be somewhat increased (M. G. Kritsman, M. V. Bavina).

The experiments indicate that the thyroid hormones influence protein metabolism. In healthy animals the influence of thyroidin brings about a decrease in protein amount in the aorta and an increase in the liver. It is probable that the thyroid hormones increase the intensity of protein disintegration in the aorta, while in the liver a compensatory increase in protein synthesis occurs at the same time. The increase in the liver fraction A corresponding to serum albumin confirms this supposition, since the albumin content of the blood serum also increases under the influence of thyroidin, while the liver is the very place where albumins are synthesized (S. Y. Kaplansky et al.; D. V. Khesin et al.). The blood proteins in this case apparently reflect the whole complex of metabolic processes within tissues.

In experimental atherosclerosis thyroidin retains in the main that direction of influence upon protein metabolism, which has been found in normal animals. In animals fed with cholesterol and thyroidin simultaneously the protein level in the aorta and the blood serum proves to be lower and in the liver higher than that in rabbits with experimental atherosclerosis.

In comparison with healthy animals, however, the protein level in the liver of rabbits fed with cholesterol and thyroidin was nevertheless reduced. Apparently, during the development of experimental atherosclerosis there occurs a reduction in the protein content of the liver, whereas administration of thyroidin simultaneously with cholesterol prevents this reduction. Thyroidin also inhibits cholesterol-induced reduction in the level of most of the soluble protein fractions in the liver and the development of hyper-beta-globulinaemia characteristic of atherosclerosis.

The present data just as the data of our previous investigations are evidence of a considerable influence produced by the thyroid hormones on the liver, through the medium of which their action on lipid and protein metabolism is apparently manifested.

The data obtained allow the conclusion that the inhibitory effect of the thyroid hormones on the development of atherosclerosis is not restricted to their favourable influence on cholesterol metabolism but extends to many aspects of the pathogenesis of the disease, including, in particular, protein metabolism.

CONCLUSIONS

1. In normal rabbits the influence of thyroidin is found to cause a decrease in the soluble protein content of the aorta and an increase in the protein content of the liver; the level of most of the soluble protein fractions in the liver also increases. The total protein content of the blood serum does not show a substantial change; there is only a slight increase in the albumin content and a decrease in γ-globulins.

2. During experimental atherosclerosis thyroidin inhibits cholesterol-induced decrease in the protein content of the liver, a decrease in the level of most of the protein fractions in the liver, and the development of hyper-beta-globulinaemia.

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FURTHER STUDIES OF THE INFLUENCE OF ASCORBIC ACID ON THE DEVELOPMENT OF EXPERIMENTAL ATHEROSCLEROSIS

L. V. KASATKINA, N. M. LOBOVA, M. I. SUKASOVA

The problem of the expediency of using ascorbic acid in atherosclerosis required further studies in view of the contradictory experimental and clinical data regarding the lipotropic action of this substance. During the last ten years a number of investigators have proved the positive influence of ascorbic acid on the course of atherosclerosis (A. L. Myasnikov; I. K. Grabenko; N. E. Kavetsky; Willis et al.).

I. A. Myasnikova (1951) discovered the inhibitive effect of ascorbic acid on experimental cholesterolaemia and lipoidosis in rabbits.

Experiments staged by Chakravarti and Mukerji demonstrated the inhibitory effect of ascorbic acid on lipoidosis in rabbits while with regard to cholesterolaemia it proved inactive. Willis and Fishman observed the appearance of atherosclerotic changes in guinea pigs kept on a cholesterol- and ascorbic acid-free diet. The same authors observed an interesting inversely proportional relation between the ascorbic acid level in different portions of the aortic intima and the degree of atherosclerotic changes in them.

At the same time, Flexner and others failed to find an inhibitory effect of ascorbic acid on cholesterolaemia and lipoidosis in rabbits (it should be pointed out that these authors used negligible doses of ascorbic acid).

K. G. Volkova also did not find an inhibitory effect of ascorbic acid on experimental atherosclerosis, using the method of extraction of total lipids from the aortic wall for estimating the degree of atherosclerotic changes.

In view of the contradictory character of literary data we undertook another study of the influence of ascorbic acid on the development of experimental lipoidosis in rabbits.

The research was carried out on 53 male Chinchilla rabbits weighing from 2.5 to 3 kg.

Atherosclerosis was induced by feeding the animals with a cholesterol suspension in sunflower-seed oil (in a dose of 0.2 g of cholesterol per kg of body weight). The experiments lasted three months and were performed in two series. In each series the animals were subdivided into two groups: a control group (kept exclusively on cholesterol food) and an experimental group (kept on cholesterol and ascorbic acid administered per os in a dose of 100 mg per kg of body weight).

Guided by the known inversely proportional relation between the functional condition of the thyroid gland and the predisposition to experimental atherosclerosis (V.V. Tatarsky; V.V. Tatarsky and V.D. Tzinzerling) before the beginning of the first series of experiments we investigated the thyroid function in rabbits according to radioiodine (I^{131}) absorption indices. The animals were given a subcutaneous injection of I^{131} in a dose of 1 μc, followed by determination of the speed and degree of I^{131} absorption by the thyroid (2 and 24 hours after isotope injection).

Each animal in the experimental group had a counterpart in the control group with an approximately equal activity of the thyroid gland. The average index of the degree of I^{131} absorption (in 24 hours) in control animals was 15.8 ± 2.6 per cent, in experimental animals—14.7 ± 2.0 per cent.

It seemed to us that under such experimental conditions it was possible to obtain a greater initial "uniformity" of control and experimental rabbits than in the case of their distribution in pairs in accordance with the degree of cholesterolaemia developed after three weeks' cholesterol feeding (as is suggested by K. G. Volkova). Indeed, this initial hypercholesterolaemia does not predetermine the animals' susceptibility to lipoidosis, the more so as no strict regularity has been established in the time and rate of cholesterolaemia increase.

During the experiment a study was made on the cholesterolaemia dynamics in rabbits (blood cholesterol was examined once in every three weeks by Abel's method) and on the ascorbic acid saturation of the blood (by dichlorophenol-indophenol titration). Upon completion of the experiment rabbits of the first group were subjected to another examination for the thyroid function by the degree of radioiodine absorption.

Comparison of the data obtained with the initial data revealed a certain decrease in the thyroid function only in control animals, which corresponds to data of N. P. Samoshkin as well as N. N. Kipshidze on the inhibitory influence of the atherosclerotic process as such on the thyroid function (average index of I^{131} absorption rate after 24 hours in the control group—10.7 ± 2 per cent, in the experimental group—16.2 ± 1.8 per cent).

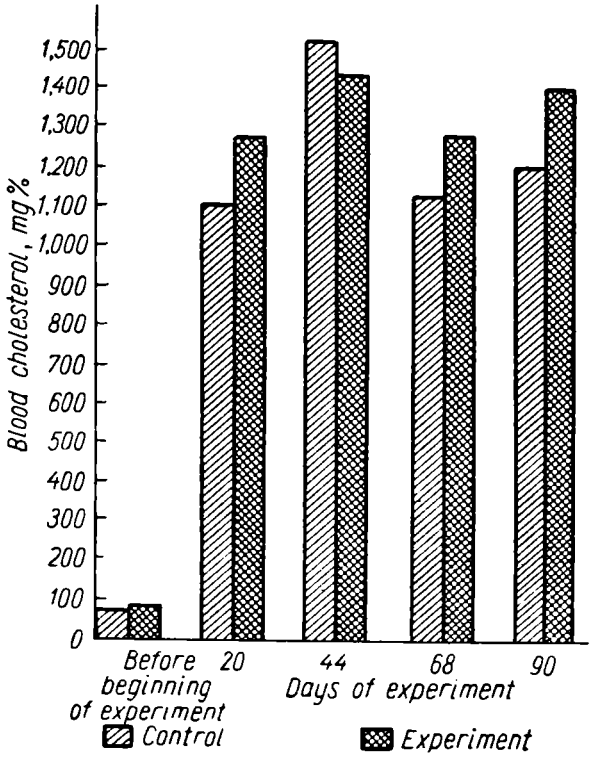

Fig. 1. Dynamics of cholesterolaemia in rabbits in the first series of experiments (average indices)

The animals were slaughtered by decapitation. Upon extraction of the aorta (the portion from the semilunar valves to the bifurcation) the degree of the visible (without special Sudan staining) sclerotic changes was determined by the indirect planimetric method after V.S. Smolensky. This was followed by extraction of the total lipids from the aortic wall by Buck and Rossiter's method. Upon removal of the adventitia, the aorta was weighed and minced. From the minced tissue were extracted lipids (at first by means of alcohol and ether, then in Soxhlet's apparatus during 8 hours) with subsequent recovery of a dry residue which was reduced to constant weight. The numerical data were processed statistically. In this way, in estimating the degree of atherosclerotic changes in the aorta we carried out a comparative study of the advantages offered by the planimetric method and by the method for total lipids extraction. The coronary arteries of rabbits were studied histologically.

A study of the cholesterolaemia dynamics in animals of the experimental and control groups in the first series of investigations revealed no influence of the ascorbic acid on the blood cholesterol level (Fig. 1), a fast rate of its increase being observed in both groups at the same time.

The average index of the cholesterol content of the blood at the end of the experiment in the control group was 1,200±99 mg per cent, in the experimental group—1,400±140 mg per cent (the difference between the mean arithmetic values was not significant, $t=1.1$).

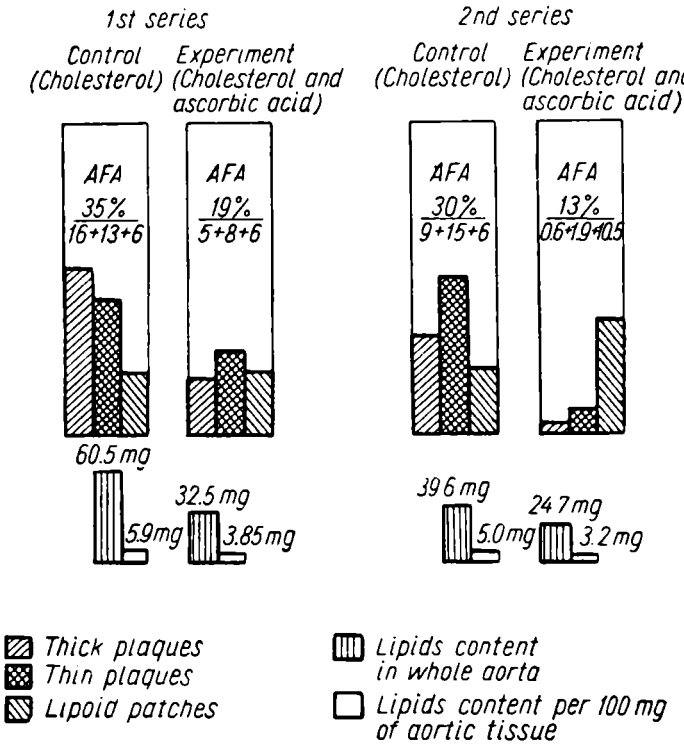

Fig. 2. Degree of lipoidosis of the aorta in rabbits. According to planimetric data and results of extraction of total lipids

The average degree of ascorbic acid saturation of the blood by the end of the experiment in the experimental group ($0.7\pm\pm0.11$ mg per cent) exceeded that in the control group ($0.4\pm\pm0.09$ mg per cent). In estimating the degree of atherosclerotic changes in the aorta of animals fed in addition to cholesterol with ascorbic acid, a distinct reduction in the degree of lipoidosis as compared to the control animals was noted (Fig. 2). The average "atherosclerotic formula of the aorta" (AFA) in the control group was $\frac{35}{16+13+6}$; in the experimental group $\frac{19}{5+8+6}$. In these formulas the numerator shows summarily (in percentage) the total aortic area occupied by atherosclerotic lesions, while the denominator consecutively gives an idea of affection by thick and thin plaques and lipoid patches. Indices obtained during extraction of the total lipids from the aortic wall also evidence a decrease in lipoidosis under the influence of ascorbic acid (see table). For example, the average index of the level of total lipids in the aorta (weight of dry residue) in experimental animals (32.5 ± 3.2 mg) proved to be considerably lower than that in the control animals (60.5 ± 5.6 mg). This difference is significant

Control group (cholesterol)			Experimental group (cholesterol + ascorbic acid)		
No. rabbit	Lipid content of whole aorta (mg)	Lipid content of 100 mg of aortic tissue	No. rabbit	Lipid content of whole aorta (mg)	Lipid content of 100 mg of aortic tissue
		First series of experiments			
2	38.9	5.25	10	42.9	17.8
3	52.7	6.58	12	10.0	2.5
4	41.7	3.16	14	70.2	22.4
5	44.2	4.60	15	37.5	58.5
6	37.8	5.50	16	27.2	7.4
7	34.9	4.92	18	26.7	11.2
8	29.7	4.30	19	23.1	8.3
9	35.0	4.28	20	39.5	18.8
11	91.4	6.8	21	32.4	16.0
13	63.6	8.19	25	21.3	5.8
17	45.1	12.40	26	50.0	49.6
22	154.6	6.62	27	34.4	18.6
23	115.5	5.24	28	22.2	11.1
24	61.5	4.85	29	12.9	3.24
			30	23.3	18.9
Average index	60.5±5.6 mg	5.9±0.6 mg	Average index	32.5±3.2 mg	3.85±0.9 mg
		Second series of experiments			
16	48.9	2.66	3	23.3	3.6
17	30.5	4.57	4	40.7	4.3
20	35.6	4.31	5	25.2	3.1
22	46.8	5.68	6	54.0	7.0
23	30.4	4.20	7	21.0	2.7
24	49.7	3.25	9	16.5	2.2
25	27.5	3.24	10	16.8	2.3
26	31.4	3.71	11	23.9	3.7
27	55.9	6.80	12	15.3	2.8
28	57.9	12.00	13	20.9	2.5
29	25.5	4.65	14	22.1	2.8
30	36.0	4.50	15	20.2	2.5
Average index	39.6±3.4 mg	5.00±0.52 mg	Average index	24.7±3.1 mg	3.2±0.25 mg

(t=3.0). Upon recalculation of the lipids deposited in the aorta per 100 mg of its raw weight, the average degree of lipid saturation of the aorta in the experimental group (3.85±0.5 mg) also proved less than that in the control group (5.9±0.6 mg).

We believe, however, that the data for the absolute lipid content of the aorta (weight of dry residue) under the conditions of the present experiment are more demonstrative than their relative level in 100 mg of aortic tissue (because the aorta was weighed in a raw state).

Thus, the first series of experiments revealed an inhibitory effect of ascorbic acid on the development of the atherosclerotic process in rabbits in spite of the absence of inhibition of cholesterolaemia. These findings are in agreement with the recently published data of M. A. Abramson who had observed a decrease of lipoidosis in rabbits under the influence of ascorbic acid without a hypocholesterolaemic effect.

The second series of experiments (24 rabbits) was undertaken for verifying the data obtained in the first series. During a study of the action of ascorbic acid on the course of experimental lipoidosis we deemed it expedient to exclude the possible influence of the seasonal factor (the first series of experiments was carried out in the spring-summer period, the second one in the autumn-winter period) as well as of the alimentary factor. The experiment was repeated on 24 rabbits (one half of the animals made up the experimental group, the other—the control group). In this case we deliberately refrained from a preliminary determination of the thyroid function in rabbits by means of radioiodine injection since the rapid increase in cholesterolaemia observed in the first series of experiments was possibly associated with a certain inhibition of the thyroid gland activity immediately after injection of radioiodine, although (as has been pointed out above) another determination of the thyroid function upon completion of the experiment revealed a moderate decrease in its activity only in control animals (which was apparently due to the influence of the atherosclerotic process itself).

In a study of the cholesterolaemia dynamics in rabbits in the second series of experiments one could note a gradual increase of cholesterolaemia and a distinct inhibition of its growth in the experimental animals which in addition to cholesterol had been fed with ascorbic acid (Fig. 3). The average index of the cholesterol content of the blood in the test animals at the end of the experiment was 800±142 mg per cent, whereas in the control animals it proved to be at the level of 1,550±222 mg per cent. This difference is significant (t=2.8). The ascorbic acid saturation of the blood in the test animals during the experiment exceeded that in the control ones (Fig. 4).

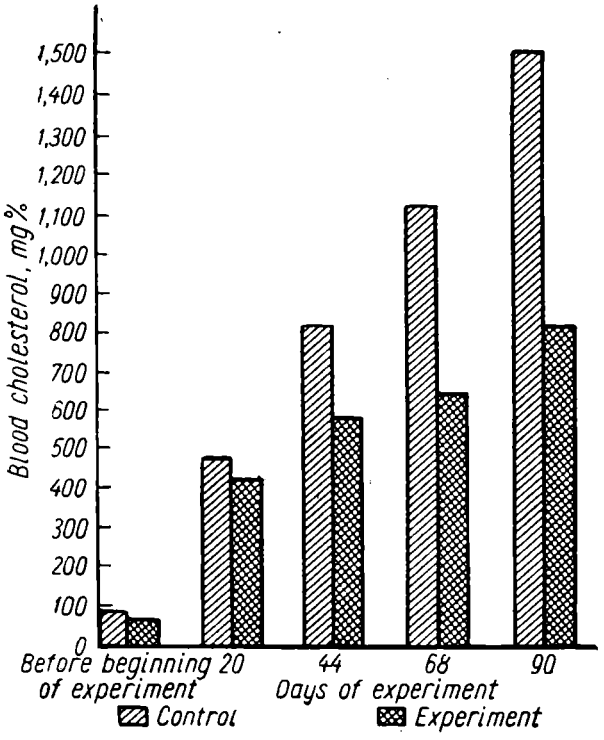

Fig. 3. Dynamics of cholesterolaemia in rabbits in the second series of experiments (average indices)

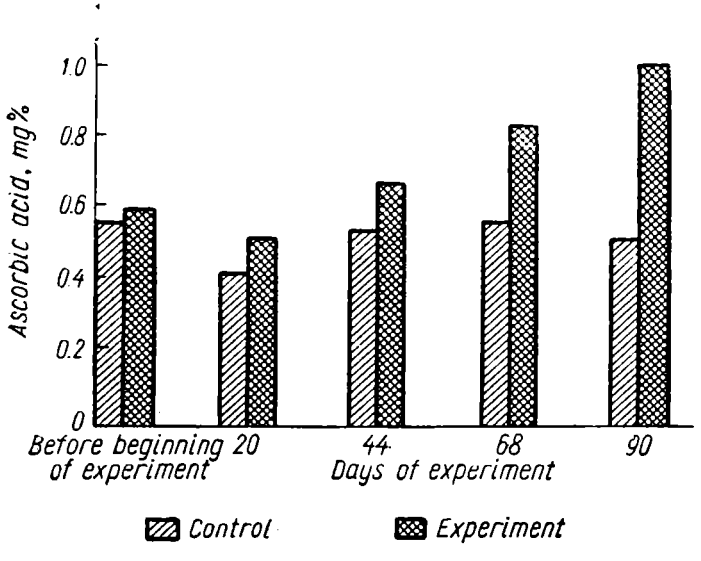

Fig. 4. The ascorbic acid content of the blood in rabbits in the second series of experiments (average indices)

A study by planimetric method revealed a distinct inhibition of the atherosclerotic process in rabbits fed with ascorbic acid in addition to cholesterol (see Fig. 2).

The average "atherosclerotic formula of the aorta" (AFA) in the control group of rabbits was $\frac{30}{9+15+6}$; in the experimental group $\frac{13}{0.6+1.9+10.5}$. Almost all experimental animals were found to have no affection of the aorta by thick atherosclerotic plaques.

The data obtained during extraction of total lipids from the aortic wall also evidence a reduction of lipoidosis in the overwhelming majority of the rabbits which in addition to cholesterol were fed with ascorbic acid (see table). Thus, the average index of the level of total lipids in the aorta of experimental animals (24.7 ± 3.1 mg) was less than that in the control group ($39.6 \pm \pm 3.4$ mg). This difference is significant ($t=2.9$). The average level of lipids in 100 mg of aortic tissue also proved lower in the experimental rabbits (3.2 ± 0.25 mg) as compared to the control group (5 ± 0.5 mg). Only two experimental animals (Nos. 4 and 6) were found to have fairly marked signs of the atherosclerotic process with a corresponding high lipid level in the aorta (see table). It is interesting that the ascorbic acid saturation of the blood in these rabbits during the experiment remained much less than that in the other animals of the experimental group. At the same time, no excessive cholesterolaemia was noted in the above-mentioned two rabbits.

Thus, the results of the second series of experiments also evidence inhibition of the atherosclerotic process in rabbits under the influence of ascorbic acid.

In estimation of the degree of atherosclerotic affection of the aorta, comparison of the indices obtained for each animal by the planimetric method and by the method of total lipids extraction in the majority of cases shows their parallelism (both in the first and second series of experiments).

Thus, the estimation of the degree of atherosclerotic changes by the planimetric method coincides in the main with that made by the method of total lipids extraction. Among the disadvantages of the latter method (apart from its complexity) is the fact that the initial level of lipids in the rabbit aorta remains unknown. According to a number of authors, this value in intact rabbits may reach 30 mg in a whole aorta or 4 mg per 100 mg of its raw weight (3.32 mg on the average). With slight signs of lipoidosis the amount of lipids in the aorta may prove close to this average index, which, naturally, complicates estimation of the data obtained.

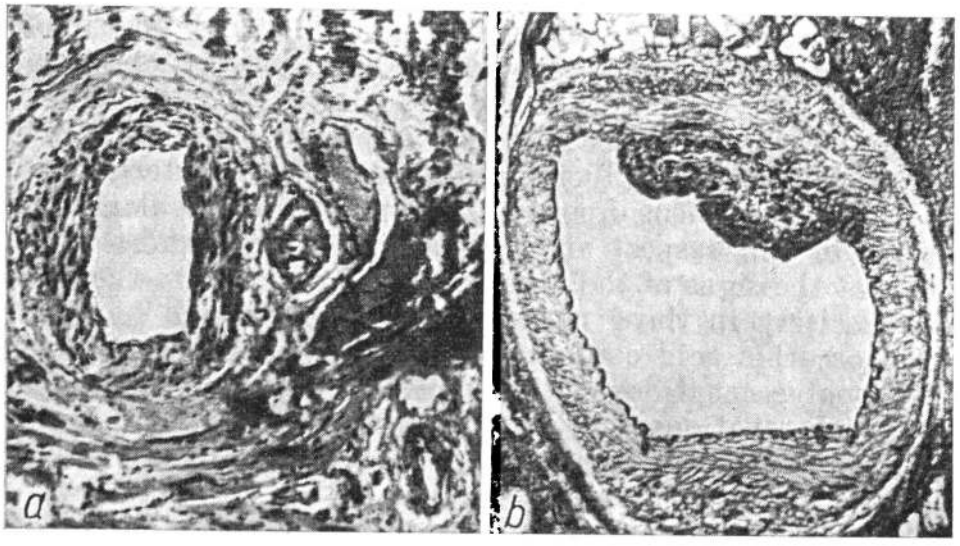

Fig. 5. Lipoidosis in the coronary arteries of rabbits (1st series). At left (*a*) experiment, at right (*b*) control. Stained after Goldman. Magnification 80 times

On the other hand, the planimetric method gives an idea of the character of lipoidosis (intensity and extension of the atherosclerotic changes), and is therefore, more advantageous.

So far as the state of the coronary arteries in animals in our experiments is concerned, the majority of the rabbits fed with ascorbic acid were not found to have a noticeable lipoidosis of these vessels, whereas in many control animals there was lipoidosis of the intramural branches of the coronary arteries, and some of them had affection of branches of a middle and large calibre attended with the formation of atherosclerotic plaques (Fig. 5).

With regard to the influence of ascorbic acid on cholesterolaemia, one can logically draw the conclusion that it produces a temporary hypocholesterolaemic effect. Such an effect was noted in the second series of experiments (whose results correspond to findings of I. A. Myasnikova). Apparently, an important role is played here by the alimentary factor and the initial ascorbic acid saturation of tissues (in case of appropriate diet in the summer-autumn period). At the same time, during a rapid increase of experimental hypercholesterolaemia this hypocholesterolaemic effect of ascorbic acid may not become manifest (which is evidenced by experiments in the first series).

The inhibition of experimental lipoidosis upon administration of ascorbic acid takes place irrespective of the degree of the developing hypercholesterolaemia and in the absence of its hypocholesterolaemic effect. This circumstance allows the sug-

gestion that the inhibitory influence of ascorbic acid on lipoidosis is effected not so much through the humoral way as through the action on the vessel wall.

Willis and Fishman showed the participation of ascorbic acid in the formation of the principal substance of the arterial wall and its inhibitory effect upon hyaluronidase. Particularly demonstrative in this respect are the data of these authors which evidence that the signs of the atherosclerotic process are the most manifest precisely in those parts of the intima which have the least local ascorbic acid content and in which depolymerization of the mucopolysaccharides of the principal substance was noted.

Clinical observations (A. V. Kostinskaya) also evidence a reduction of vascular permeability upon administration of ascorbic acid to atherosclerotic patients.

There is reason to suppose that the inhibitory action of ascorbic acid on experimental lipoidosis is realized mainly through a decrease in the permeability of the arterial wall.

CONCLUSION

During induction of experimental atherosclerosis in rabbits, administration of ascorbic acid with food (in a dose of 100 mg per kg of body weight) leads to a distinct inhibition of the atherosclerotic process, producing a changeable hypocholesterolaemic effect. A decrease in the degree of lipoidosis in rabbits under the influence of ascorbic acid is seen also in the absence of the hypocholesterolaemic effect.

The latter circumstance permits the suggestion that the mechanism of the above-mentioned action of ascorbic acid is associated mainly with a decrease in the permeability of the arterial wall.

The method for estimating the degree of atherosclerotic changes in the aorta by the amount of total lipids extracted from its walls has no special advantages over the simpler and more accessible planimetric method.

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THE INFLUENCE OF ADRENALINE AND NORADRENALINE ON THE STATE OF THE HEART MUSCLE IN CHOLESTEROL ATHEROSCLEROSIS

A. P. ZYSKO

At the present time the literature contains considerable information to the effect that catecholamine administration causes dystrophic changes in the heart muscle and microfocal necroses (Raab).

K. M. Danilova showed that even a brief administration of large catecholamine doses to rats causes dystrophic changes in the myocardium.

Since the experiments were carried out on healthy animals, it was interesting to find out the influence of catecholamines exerted upon the state of the heart muscle in atherosclerosis when the blood supply to the myocardium proves to be inadequate due to affection of the coronary vessels with lipoidosis.

The study was made on 67 Chinchilla rabbits weighing from 2 to 2.5 kg. During the experiment the cholesterol level in the blood serum was determined by Engelgardt-Smirnova's method once in every two weeks. The arterial pressure in the carotid artery drawn out onto a skin flap was measured every other day. At the end of the experiment an electrocardiogram was taken for all of the rabbits, while the experimental rabbits which in addition to cholesterol were fed with adrenaline or noradrenaline were subjected to electrocardiography before and after the administration of these preparations. Upon completion of the experiment the animals were slaughtered, and the aorta and heart subjected to morphological examination.

From each heart 6-9 sections were taken and stained with hematoxilin-eosin, Sudan III and sometimes with fuchsiline for elastic fibres.

The experiments were carried out in three series lasting 9, 16 and 12 weeks. In the first two series the object of study was the influence of noradrenaline injected to rabbits intramuscularly in a dose of 100 γ every other day (first series) and daily (second series). In the third series a study

was made on the influence of adrenaline injected to animals intramuscularly in a dose of 200 γ daily.

Atherosclerosis was induced by means of feeding the animals with cholesterol dissolved in sunflower-seed oil in the following doses: in the first series 0.2 g per kg of body weight; in the second and third series 0.1 g per kg of body weight. In each series of experiments the animals were subdivided into three groups. The animals in the experimental group were given cholesterol and adrenaline or noradrenaline; the animals in the control group were given only cholesterol; the rabbits in the third group were given only adrenaline or noradrenaline.

The level of the arterial pressure in the rabbits given cholesterol and noradrenaline or adrenaline intramuscularly as well as in the animals given only noradrenaline or adrenaline intramuscularly was higher during the experiment than that in the animals of the control group by only 10-15 mm Hg, i.e., no difference in pressure was in effect noted. A single intravenous injection of noradrenaline to 9 rabbits was noted to be followed by an increase in the arterial pressure by 20-55 mm Hg, 35 mm Hg on the average. Upon intravenous injection of adrenaline to four rabbits the arterial pressure increased by 30-35 mm Hg. Upon completion of the infusion the arterial pressure returned to the initial level as early as in five minutes' time and sometimes fell to a lower level.

The study of the ECG changes under the influence of a single injection of noradrenaline was carried out in 14 rabbits to 7 of which noradrenaline was injected in a dose of 100 γ, and to the remaining seven in a dose of 200 γ. Intravenous injection of adrenaline was given to 20 animals, of which one rabbit was given a dose of 100 γ, and the others a dose of 200 γ.

Intravenous injections of catecholamines were immediately followed by ECG changes which usually began with sinus bradycardia followed by disorders in the rhythm and in certain cases by signs of myocardial ischaemia. Sinus bradycardia was sometimes very marked, the rate of cardiac contractions at the same time reducing 3-4 times. The most frequent type of arrhythmia was extrasystole (atrial, ventricular, polytopical, grouped, and sometimes of the bigeminal type). The ectopic rhythm was rather frequently observed during a certain length of time. In one case, adrenaline administration was in five minutes' time followed by ventricular fibrillation and death (Fig. 1). Extrasystole and the ectopic rhythm more often took place upon administration of adrenaline rather than noradrenaline. Administration of noradrenaline was usually followed only by sinus bradycardia, sometimes accompanied by extrasystole. Arrhythmia was often accompanied by a delay in the intraventricular conduction to the degree of blocking the peduncles of His' bundle.

The ectopic rhythm was sometimes replaced by a monophasic

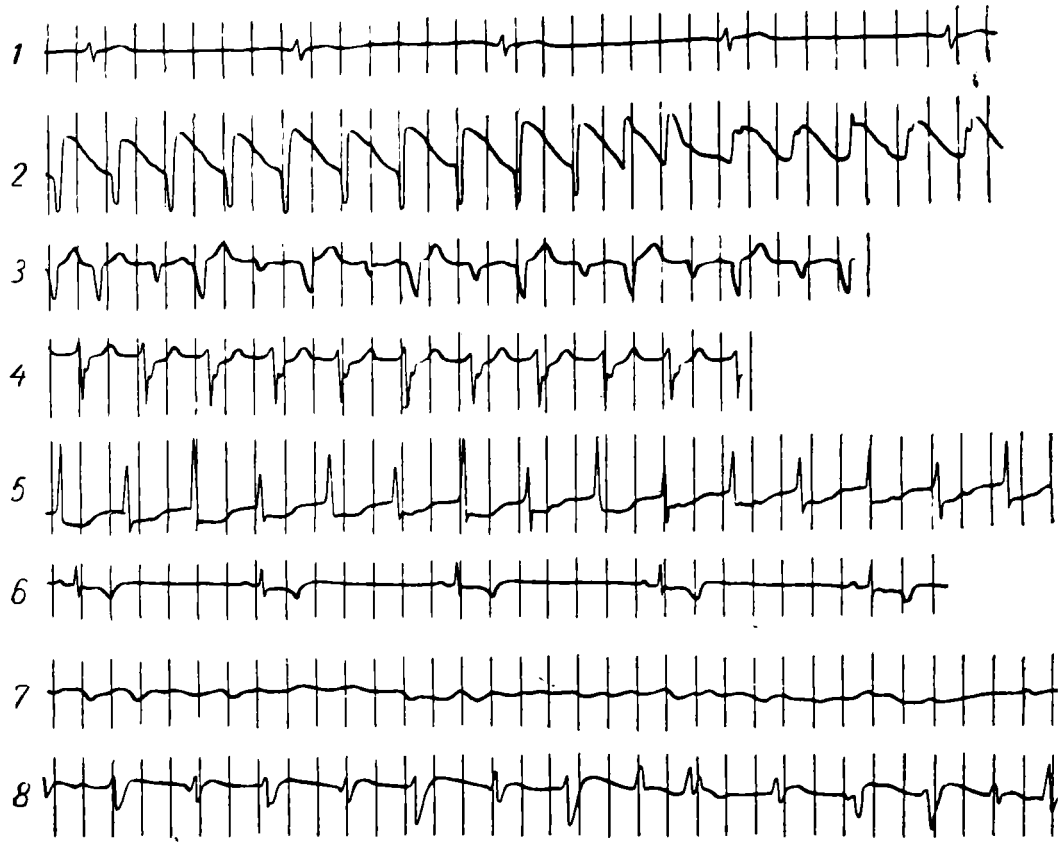

Fig. 1. Disturbances in rhythm and conductivity observed during intravenous injection of adrenaline and noradrenaline to rabbits.

1—sinusal bradycardia; *2*—ectopic rhythm with transition to monophasic ischaemic curve; *3*—bigeminia; *4*—nodular rhythm; *5*—electrical alternation of the heart; *6*—sinusal bradycardia and myocardial ischaemia; *7*—ventricular fibrillation; *8*—extrasystole, intraventricular block

ischaemia curve, but more often the signs of myocardial hypoxia were noted in 3 to 5 minutes' time after the injection of catecholamines and were manifested by a discordant displacement of the *ST* interval in the standard leads: lead *I* showed an arch-shaped elevation, lead *III* a reduction. A frequent increase in the *ST* interval was also noted in lead V_4 (Fig. 2). In certain cases the *ST* interval remained on the isoelectric line, but a negative *T* wave appeared in leads *I* and *II* and in V_4. ECG changes pointing to myocardial hypoxia were noted for 5-10 minutes after the infusion of catecholamines and only in individual cases persisted for a longer time.

ECG signs of myocardial ischaemia upon administration of adrenaline appeared in 14 rabbits out of 20; upon administration of noradrenaline in 5 animals out of 14. In one rabbit intravenous injection of 200 γ adrenaline produced a typical mon-

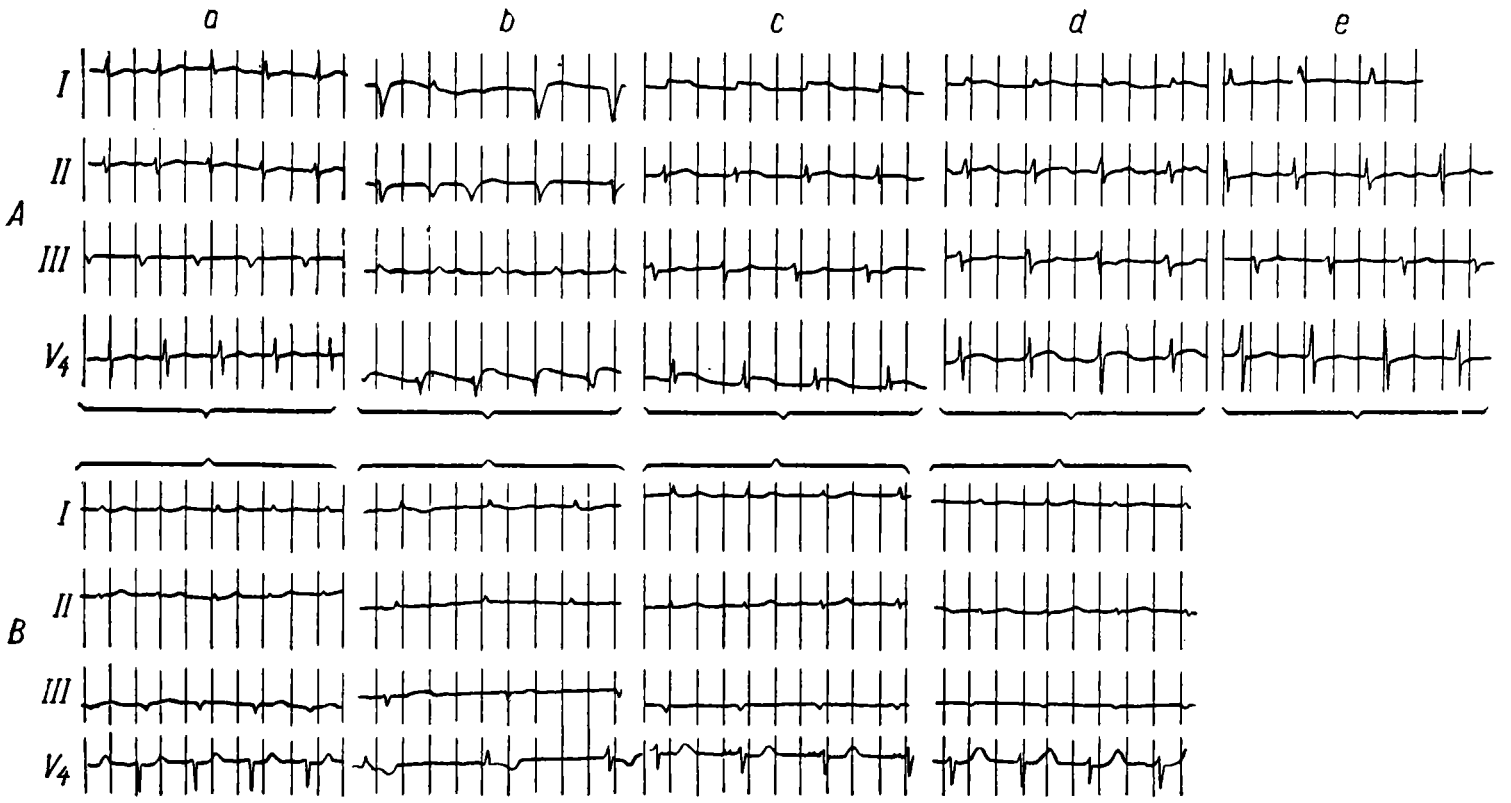

Fig. 2. The most frequent succession of ECG changes in rabbits during intravenous adrenaline and noradrenaline injections.

A—adrenaline injection; *B*—noradrenaline injection; *a*—initial ECG; *b*—ECG immediately upon injection; *c*—ECG after 5 min; *d*—ECG after 10 min; *e*—ECG after 30 min

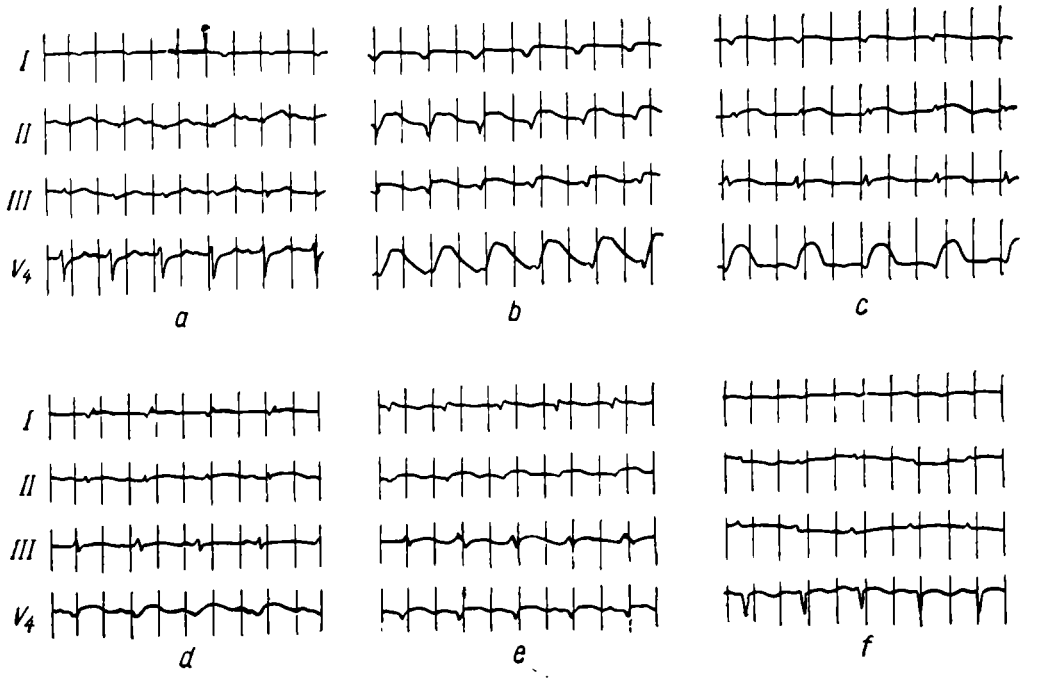

Fig. 3. ECG dynamics in rabbit No. 37 during adrenaline injection.

a—initial ECG; *b*—ECG immediately upon injection; *c*—ECG after 5 min; *d*—ECG after 30 min; *e*—ECG after 24 hours; *f*—ECG after a week

ophasic ischaemic curve: R wave disappeared, QS interval appeared in lead V_4, the ST interval was markedly elevated and arch-shaped. In 24 hours' time the ST interval was still slightly elevated, then it declined to the isoelectric line; there appeared a negative T wave in lead V_4, QS complex remaining in lead V_4. These changes were noted during a week (Fig. 3). Histological examination of the heart of this rabbit revealed an extensive cicatricial area 8×3 mm in size in the depth of the left ventricle.

In the first series of experiments made on 18 rabbits of which 8 were given cholesterol and adrenaline, 8 only cholesterol and 2 only noradrenaline, all animals given cholesterol and noradrenaline had signs of oxyphilic dystrophy of the myocardium and one rabbit had a slight microfocal cardiosclerosis.

In the second series of experiments made on 14 animals of which 6 constituted the control group, 7 were given cholesterol and noradrenaline and 1 only noradrenaline, there was a marked difference in the state of the heart muscle in rabbits of the control group given only cholesterol and in those of the experimental group given cholesterol and noradrenaline. In the control group the muscle fibres had a normal structure and took a stain readily and uniformly (Fig. 4), whereas in the control group one could note signs of marked oxyphilic and fatty dystrophy

of the myocardium: the fibres had blurred outlines and were ununiformly stained with hematoxylin-eosin. Stained with Sudan they showed a large amount of fat. Five of seven rabbits in this group had microfocal cardiosclerosis: there were small foci of connective scar-tissue between muscle fibres and in the place of dead fibres (Fig. 5).

Some rabbits were found to have small subendocardial necroses and thickening of the endocardium. In certain cases there were fresh haemorrhages in the perivascular tissue. The foci of atherosclerosis were usually located in the subendocardial region but in many cases were also encountered in other regions of the heart. The left ventricle was affected most frequently.

The influence of adrenaline on the degree of cholesterolaemia and the state of the heart muscle was studied on 35 rabbits in the third series of experiments. The control group consisted of 13 rabbits which were given exclusively cholesterol. The experimental group was made up of 19 animals which were fed with cholesterol and in addition were given daily adrenaline. Three rabbits were given adrenaline alone.

Histological examination of the heart muscle revealed a marked eosinophilic and fatty degeneration of the muscle fibres in the group of rabbits given cholesterol and adrenaline. In this group 13 rabbits were found to have microfocal cardiosclerosis as well as small foci of necrosis and haemorrhages in the heart muscle, mainly in the subendocardial region of the myocardium. In addition, two rabbits in this group were found to have large foci of necrosis histologically resembling cicatrizing myocardial infarction (Fig. 6). In one of these rabbits a large necrotic focus 5×3 mm in size was located in the papillary muscle of the left ventricle. There were dead muscle fibres in the centre of the region and developing granulation tissue on the periphery (Fig. 7). The maximum level of cholesterol in this rabbit reached 949 mg per cent, lipoidosis of the coronary arteries was insignificant. Another rabbit, also with slight lipoidosis of the coronary arteries and a maximum cholesterol level of 936 mg per cent in the blood serum was found to have two sizable foci of necrosis 3×3 mm in size in the papillary muscles of the left ventricle and several smaller necrotic foci in the subendocardial region.

Histological examination of the heart in one of the rabbits in this group revealed an extensive area of connective scar-tissue in the site of former necrosis located in the depth of the muscle of the left ventricle and extending to the surface of the heart (in isolated sections) (Fig. 8). The size of this region was 8×3 mm. The maximum cholesterol level in this rabbit reached 1,287 mg per cent. Lipoidosis of the coronary arteries was fairly marked. It was made quite clear by electrocardiography that the emer-

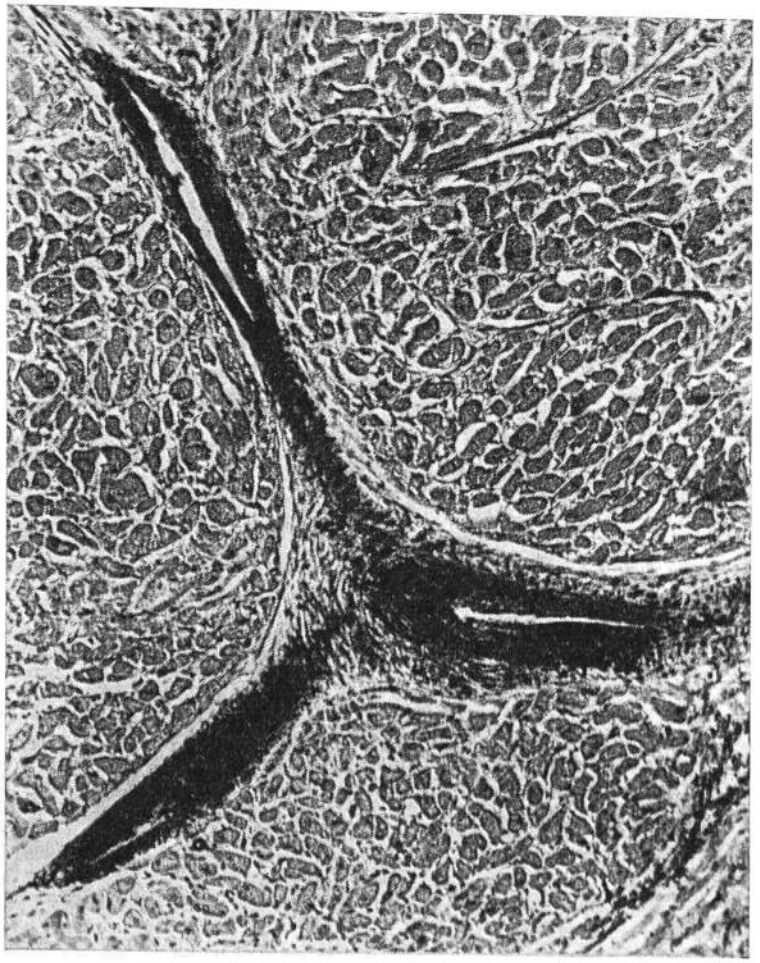

Fig. 4. Normal structure of muscle fibres of the myocardium in a control group rabbit

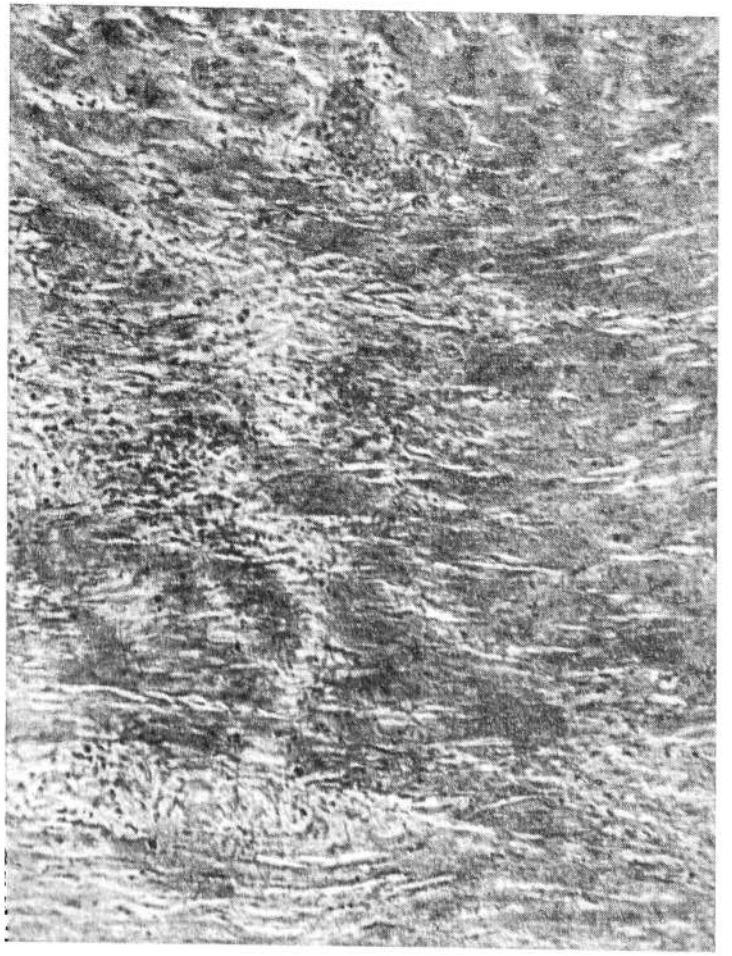

Fig. 5. Microfocal cardiosclerosis. Myocardial dystrophy

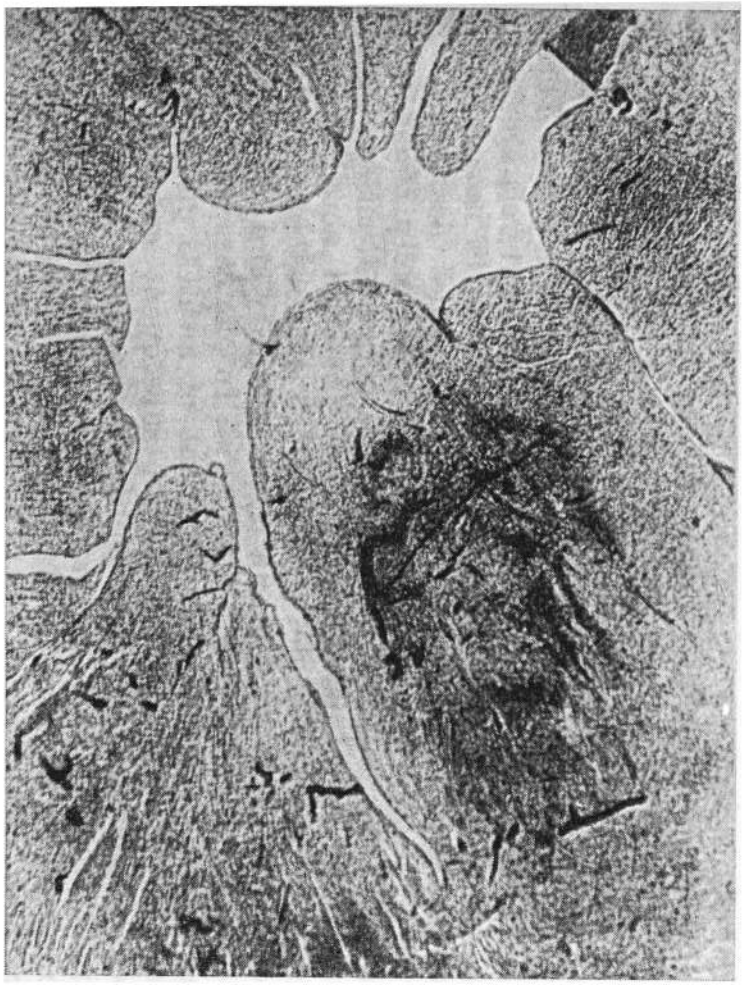

Fig. 6. A large area of necrosis in the papillary muscle of the rabbit heart

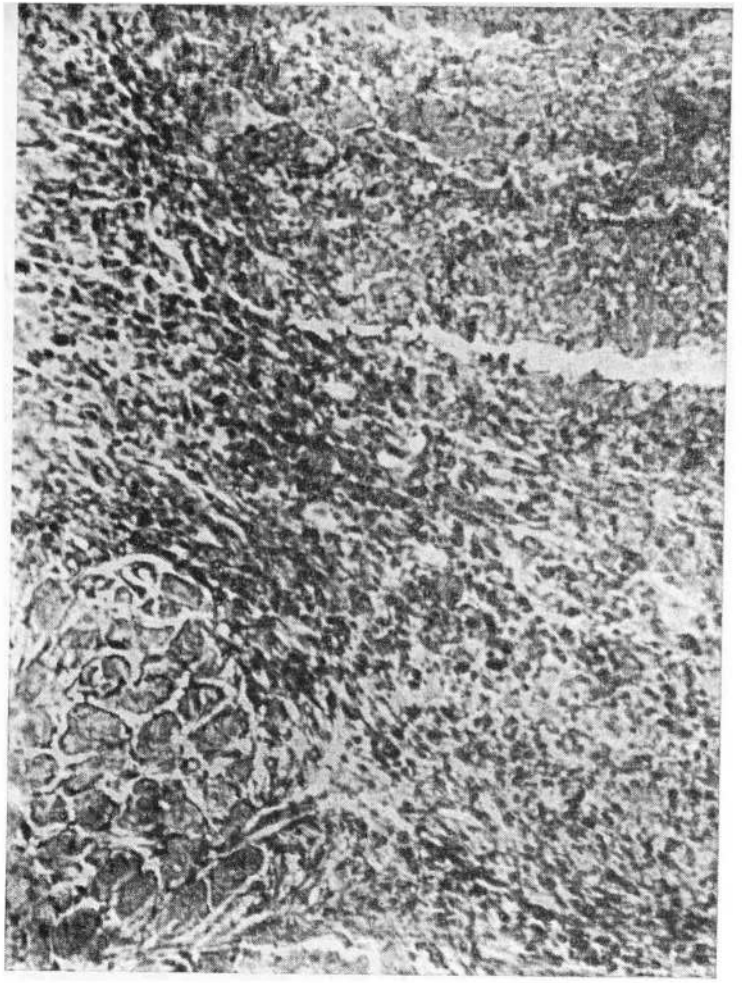

Fig. 7. Macrofocal necrosis of the myocardium (high-degree magnification). At right upper corner—dead muscle fibres; in centre—granulation tissue; at left lower corner—normal muscle fibres

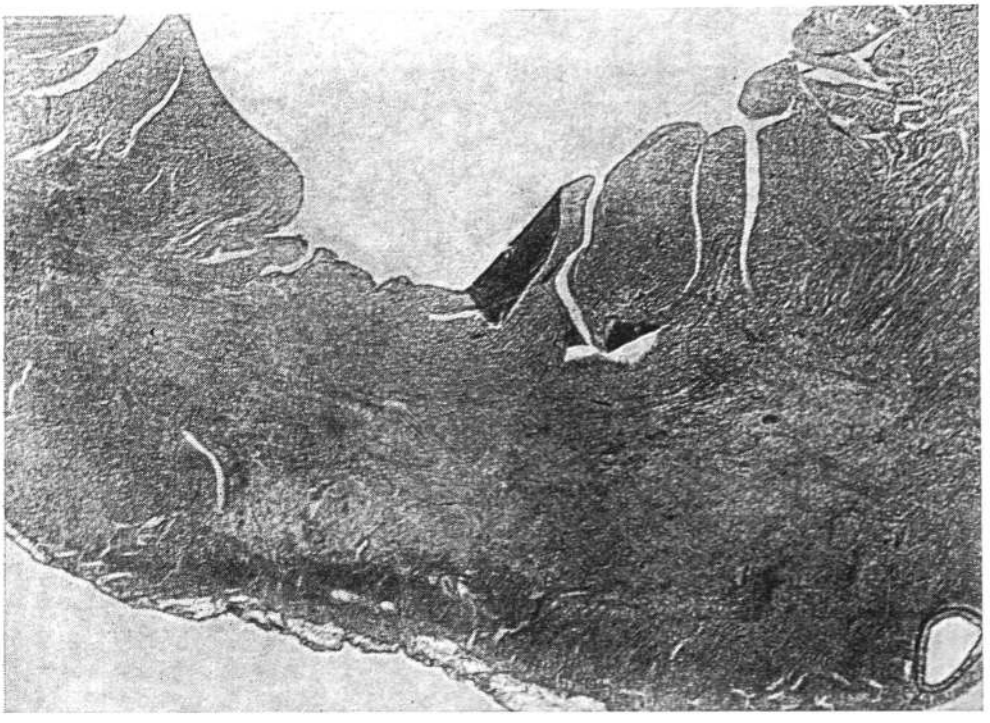

Fig. 8. A large scar in the depth of the muscle of the left ventricle

gence of a necrotic focus in this rabbit was associated with adrenaline administration.

Six rabbits with focal changes in the myocardium showed signs of pericarditis. One could see a slightly red opalescent fluid in the pericardium. The epicardium was thickened, oedematous, infiltrated by leukocytes; at places there were haemorrhages with broken-down erythrocytes, and fibrin deposits. Three rabbits to which noradrenaline had been administered and two rabbits out of three given adrenaline were found to have microcellular cardiosclerosis and small subendocardial necroses.

In the control group of rabbits given exclusively cholesterol two animals were found to have slight microfocal atherosclerosis.

In this way, administration of adrenaline and noradrenaline to rabbits kept on a cholesterol diet led to considerable ECG changes pointing to disorders in the excitability and conductivity of the heart muscle and its hypoxia. In one case, adrenaline administration caused the development of an ECG picture typical of myocardial infarction with subsequent characteristic changes. Disturbances of the rhythm were more often noted upon injection of adrenaline than noradrenaline. Analogous data were obtained by Lepeschkin; Raab (1953); I. K. Shkhvatsabaya. Disturbances in the excitability and conductivity noted upon

administration of catecholamines were possibly associated with ischaemia of the myocardium the presence of which was evidenced by electrocardiographic and histological changes.

Changes in the heart muscle discovered in the group of animals to which adrenaline was given in addition to cholesterol deserve notice. In addition to the dystrophic changes and microfocal necroses described by Raab, K. M. Danilova and other authors we have discovered in two cases large (rather than small coalesced) necrotic foci histologically typical of myocardial infarction, and in one case a large scar in the depth of the left ventricular muscle. In Raab's opinion, catecholamines cause necrobiotic changes owing to a sharp increase in the oxygen consumption of the myocardium, which has been proved by Golwitzer-Meier and others. During atherosclerosis the coronary vasodilatation proves insufficient, and the administration of adrenaline and noradrenaline produces anoxia as a result of exhaustion of the oxygen reserves in the coronary arteries (Raab). This may account for the appearance of large and small necrotic foci in our experiments also. The great frequency of necrobiotic changes in the group of rabbits given adrenaline in addition to cholesterol as compared to another group of animals to which noradrenaline had been administered is apparently due to the fact that adrenaline caused a considerably larger increase in the oxygen consumption of the myocardium than did noradrenaline; this was possibly because the dose of adrenaline in our experiments was twice as large as that of noradrenaline.

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THE INFLUENCE ON THE HEART OF STIMULATION OF THE CEREBRAL LATERAL VENTRICLES IN THE RABBIT IN EXPERIMENTAL ATHEROSCLEROSIS

I. K. SHKHVATSABAYA, Y. V. POSTNOV

The present research continues the cycle of investigations which have for their aim to ascertain the significance of the nervous mechanisms in the development of acute affections of the heart.

Our previous experiments made it clear to us that stimulation of the central nervous system by blowing air into a lateral ventricle is extremely powerful and is accompanied by a rapid appearance of pathological changes in the cardiovascular system of the experimental animal, which is corroborated by electrocardiographic and morphological data. These experiments have shown, in particular, that morphological changes in the heart are associated mainly with disorders in the myocardial blood and lymph circulation. An additional investigation with the use of neurotropic pharmacological substances and a quantitative determination of catecholamines in the myocardium and adrenals of experimental rabbits allowed the supposition that the above-mentioned changes in the cardiovascular system had been caused by an excessive activation of the sympathico-adrenal system occurring in response to the nervous stimulation noted above.

The task we set ourselves in the present experiment was to study changes in the heart in rabbits with induced atherosclerosis in response to acute nervous stimulation. We proceeded from the assumption that among the factors responsible for the development of an acute disturbance of the coronary circulation and myocardial infarction a considerable role is played by coronary atherosclerosis and derangement of the nervous regulation of the cardiac activity and coronary arteries. It was these two conditions that were reproduced in the given experiments.

The methods of the experiment were as follows. The rabbits were fed with large doses of cholesterol (1 g per day) during periods varying in length from 98 to 158 days. Upon completion of cholesterol feeding, the animals were subjected to acute nervous stimulation by blowing air into the lateral ventricle, whereupon they were slaughtered or died after different periods of time (from 15 minutes to 7 days after stimulation). The experiments were made on ten rabbits. The details of the methods applied for stimulating the cerebral ventricles are omitted, since these findings were published earlier (I.K. Shkhvatsabaya, 1961).

The control group consisted of five rabbits which in analogous periods were fed with cholesterol and then slaughtered.

For a morphological study the heart was extracted, fixed with a 10 per cent neutral formalin (formaldehyde) solution. Part of the material was preserved with paraffin, the remainder being treated on a freezing microtome. The following stains were used: hematoxylin-eosin, Sudan III, Masson's stain.

A systematic study of the electrocardiogram of cholesterol-fed rabbits showed that ECG changes in these animals were

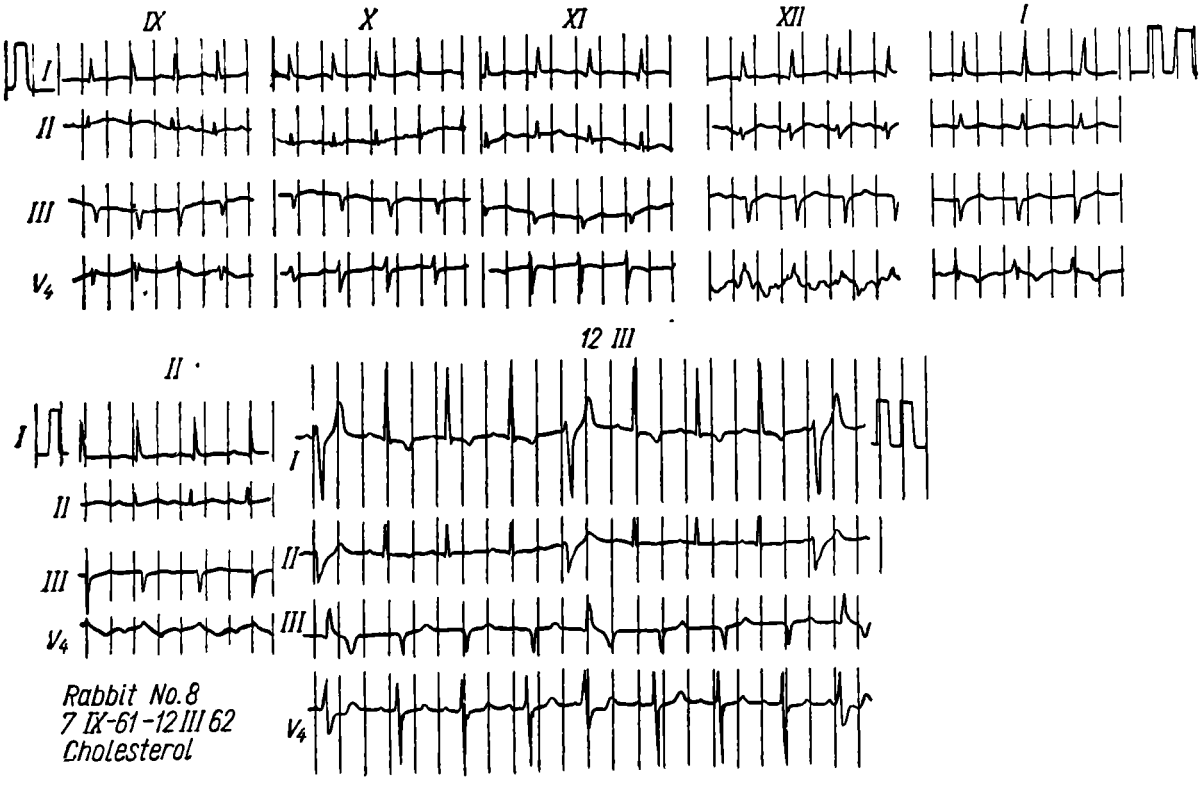

Fig. 1. Electrocardiogram of a rabbit kept on a cholesterol diet during 7 months (consecutive monthly ECG data).

Gradual increase in voltage of R_1 wave, appearance of negative T_{I-II} wave and ventricular extrasystoles in the 7th month of experiment (March 12, 1962). ECG was taken in three standard leads and in fourth thoracic lead

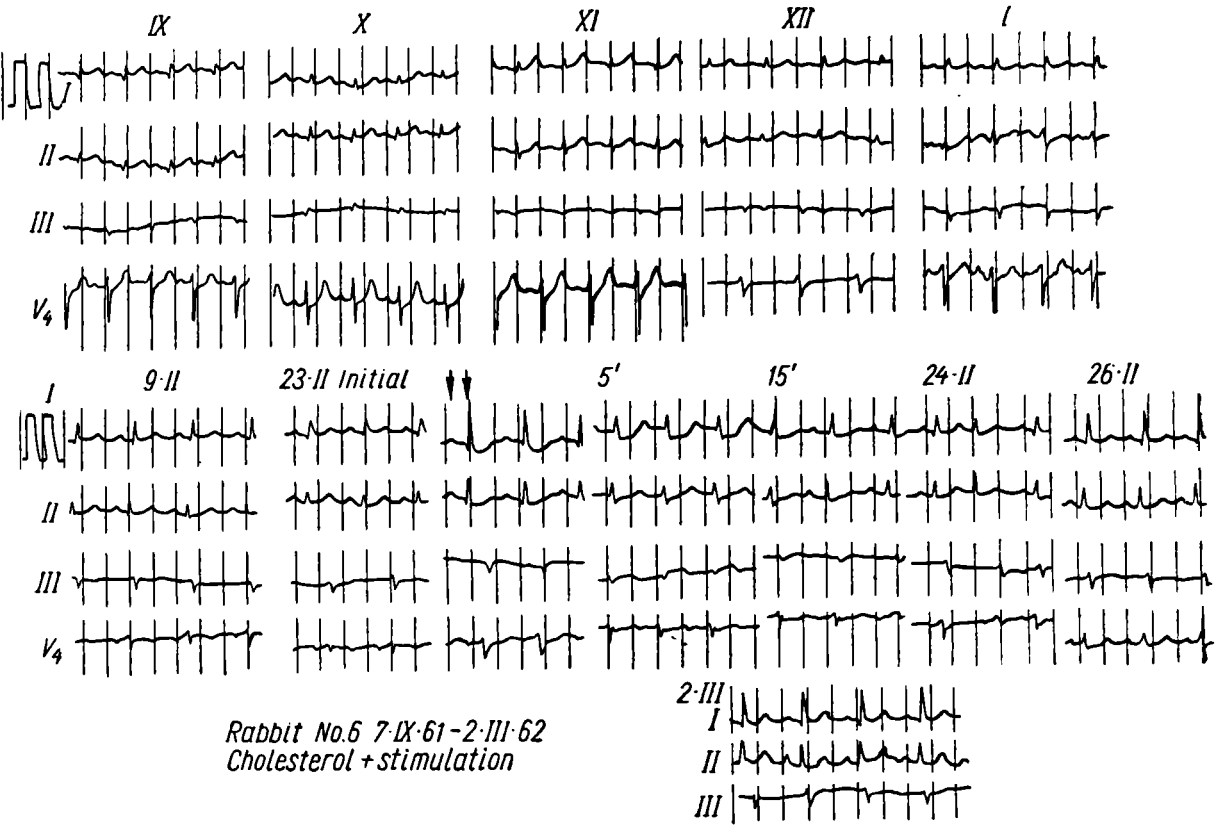

Fig. 2. Electrocardiograms of a rabbit kept on a cholesterol diet during 6 months and subjected to a nervous stimulation (consecutive monthly ECG data).

Increase in voltage of R_1 wave during cholesterol feeding. Ischaemic ECG changes with subsequent dynamics after a nervous stimulation applied on February 23, 1962 (moment of stimulation is indicated by arrows). ECG was performed in three standard leads and fourth thoracic lead

quite insignificant, being confined mainly to changes in the voltage of R wave without noticeable changes in the configuration of the complexes and waves even during prolonged cholesterol feeding. Only in one rabbit the voltage of R wave began to increase gradually from the 4th month of cholesterol feeding, a reduction of the ST_1 segment was noted below the isoline, and on the 7th month there occurred inversion of T_1 wave and frequent ventricular extrasystoles which periodically appeared in groups, sometimes of the allorhythmic type (Fig. 1).

Injection of air into the lateral ventricle quickly led to ECG changes like those we had observed during preceding investigations in animals kept on a cholesterol-free diet (Fig. 2). Usu-

ally, bradycardia appeared at the moment of air injection; then, if the stimulation was strong enough, a ventricular paroxysmal extrasystole developed which ended in grouped and isolated extrasystoles, including polytopical ones, and finally there developed changes in the *ST* segment—its displacement below the isoline in lead *I* and an arch-shaped elevation in lead *III*. These curves resembled monophasic curves in shape and could be evidence of an acute myocardial ischaemia. It should be noted that after nervous stimulation the survival rate among rabbits with experimental atherosclerosis was noticeably less than in rabbits which had not been given cholesterol. For example, of the ten rabbits with induced atherosclerosis in the given experiment five perished within 15 to 150 minutes even after a single stimulation whereas in an earlier study only two rabbits died $2^1/_2$ hours after injection of air into the lateral ventricle out of 20 rabbits which had not been given cholesterol. The comparison adduced above is unquestionable evidence of a reduction in the adaptational capacity of animals with experimental atherosclerosis to nervous stimulation.

Turning to the characteristics of morphological changes in the heart of rabbits in the control and experimental groups we believe it expedient to begin with a description of the morphological changes which were common for the rabbits in both groups.

The rabbits in both groups had marked atherosclerosis of the aorta designated with 3-4 crosses (under the four-point system of estimating the degree of aortic affection) and also just as marked lipoidosis of the intramural branches of the coronary arteries. In branches of a larger calibre lipoidosis was not found.

In both groups of animals the myocardium showed dystrophic changes manifested by a granular swelling of the muscle fibres, loss of striation, irregular staining of the fibres attended with accumulation of free lipids in the sarcoplasm. These signs might be qualified as a manifestation of protein-lipid dystrophy of the myocardium. They were noted in isolated muscle fibres and groups thereof and sometimes were of a diffuse character, being confined, however, mainly to the subendocardial layers of the myocardium. In addition to this, foci of cardiosclerosis were discovered in animals of both groups.

For rabbits with experimental atherosclerosis subjected to nervous stimulation very characteristic, in addition to the above-described changes, was the presence of distinct symptoms of an acute disturbance of the blood and lymph circulation in the myocardium in the form of a paretic dilatation of smaller vessels (arterioles, venules and capillaries) with the development of venous and capillary stases and lymphostases with lymphorrha-

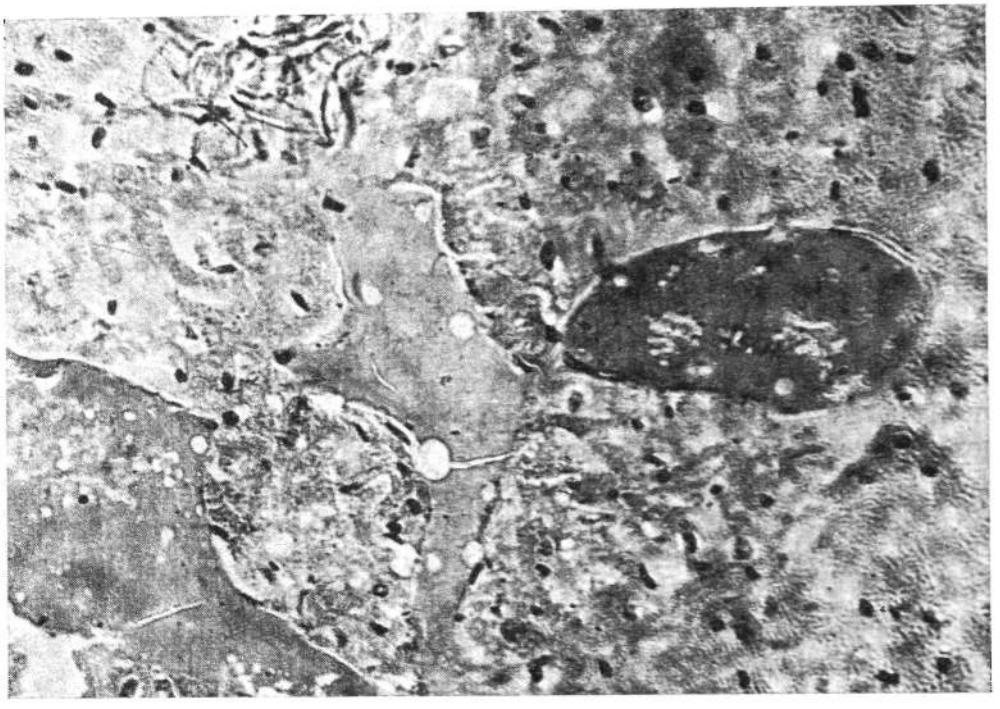

Fig. 3. Micropreparation of a portion of the myocardium of the left ventricle of the rabbit heart (atherosclerosis plus nervous stimulation). Magnification 300 times. Lymphostasis, thrombi (hyaline type) in dilated veins

gia into the stroma. All these manifestations in combination with oedema of the stroma presented a peculiar picture of "bogged-up" tissue evidencing a sharp increase in the permeability of the vascular system of the myocardium (Figs. 3, 4). Diapedetic haemorrhages from the veins and capillaries were observed invariably. In addition to this, 3 rabbits of the experimental group showed the formation in the myocardium of arterial and venous thrombi (spodogenous, hyaline-type thrombi); in one case there was thrombosis of a medium-calibre artery with proliferation of the intimal cells and inclusion into the thrombus mass of fibrin, segmentonuclear leukocytes, and desquamated endothelium, which on the whole pointed to a fairly long existence of the thrombus and the beginning of its organization (Fig. 5).

The appearance of vascular thrombi in the myocardium deserves notice in view of the fact that according to data of N. N. Anichkov's school, in experimental cholesterol atherosclerosis (as distinct from human pathology), predisposition to thrombus formation in the coronary arteries is absent altogether.

In the heart of rabbits of the principal group slaughtered at late periods after nervous stimulation small disseminated focal

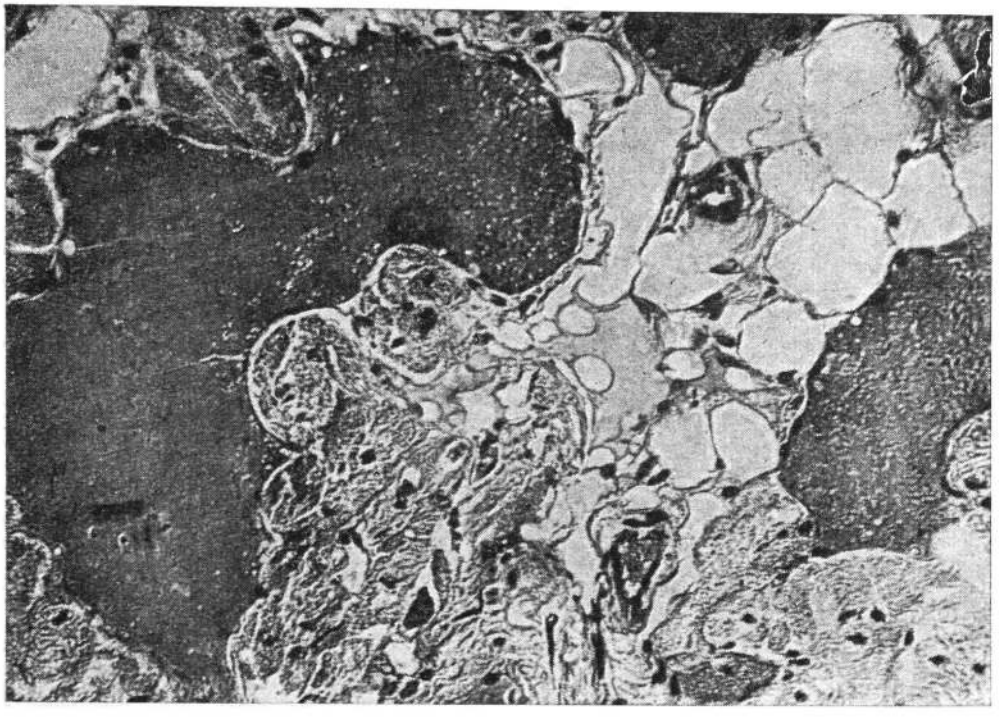

Fig. 4. Subepicardial regions of the myocardium in a rabbit (atherosclerosis plus nervous stimulation). Magnification 300 times. Venous stasis, hyaline thrombi in small veins. Lymphostasis with lymphorrhagia

necroses of muscle fibres with lysis, vacuolization and fragmentation of them were discovered along with acute circulatory disorders. The development of necrotic and necrobiotic changes in the muscle fibres was accompanied by oedema, loose leukocytic infiltration of the stroma, and diffuse productive histiocytic reaction in it (Fig. 6). Acute dystrophic changes of the muscle fibres were the most severe in the zones with the most pronounced circulatory disorders.

Such markedness of the dystrophic and necrobiotic changes in the myocardium as well as the formation of thrombi was not encountered in the earlier experiments with injection of air into the lateral ventricle in normal rabbits (I. K. Shkhvatsabaya, 1961).

The results of this experiment prompt the following three principal conclusions:

(1) acute nervous stimulation in rabbits with experimental atherosclerosis leads to sharp disturbances in the blood and lymph circulation primarily due to paretic changes in the vascular system of the heart and accompanied by an increased permeability of the vessel walls;

Fig. 5. Micropreparation of the heart of a rabbit with experimental atherosclerosis subjected to a nervous stimulation. Magnification 450 times. Arterial thrombus in a medium-calibre artery. In the mass of the thrombus are seen fibrin, leukocytes, desquamated endothelial cells. Early stage of organization

(2) acute disturbance of the innervation mechanisms of the heart in experimental atherosclerosis provides the conditions for the development of coronary thrombosis;

(3) the resistance of rabbits to nervous stimulation in experimental atherosclerosis is reduced which is manifested by a shorter term of survival of rabbits after injection of air into the lateral ventricle.

The findings adduced here point to the great role played by disturbances in the blood and lymph circulation in the pathogenesis of neurogenous affections of the heart and to the importance for this process of the paretic state of the vascular system of the heart. These data agree, in particular, with the findings of S.S. Weil (1960).

Induction of coronary thrombosis in rabbits with experimental atherosclerosis under the conditions of acute nervous stimulation is of a fundamental significance and requires further study. This also deserves attention in view of the fact that according to data of N.N. Anichkov's school, in experimental cholesterol atherosclerosis, as distinct from human pathology,

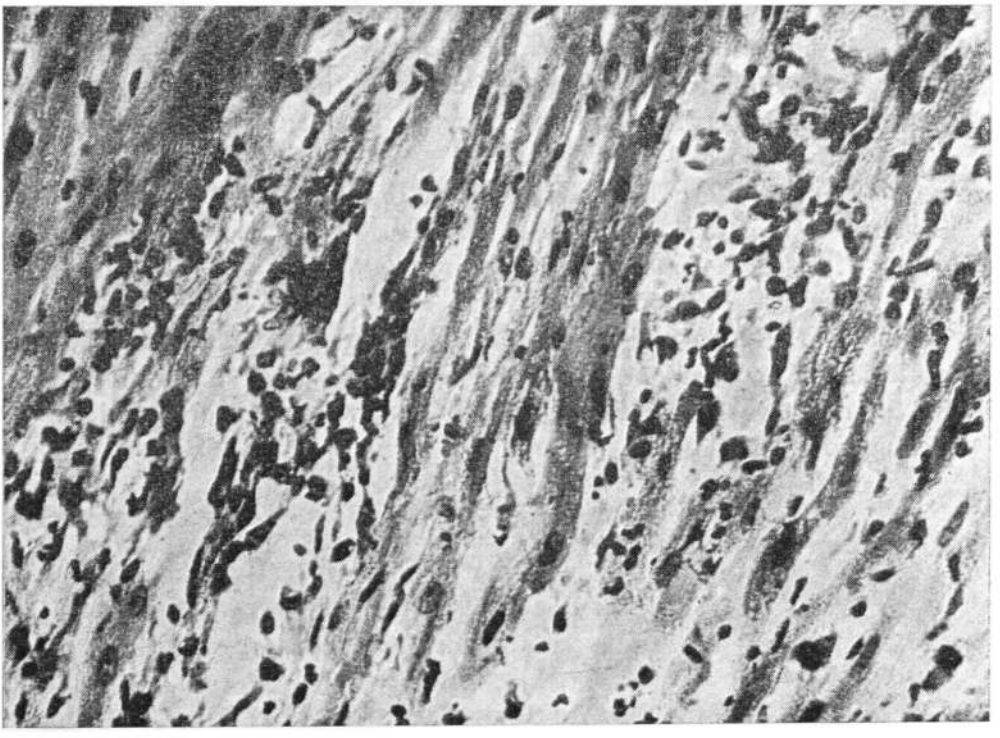

Fig. 6. Micropreparation of the rabbit heart with experimental atherosclerosis, slaughtered 4 days after a nervous stimulation. Magnification 450 times. Fine necroses of the muscle fibres, lysis of fibres, proliferative reaction of histiocytic cellular elements. Oedema of the stroma

predisposition to thrombus formation in the coronary arteries is absent. The latter circumstance is advanced as one of the principal objections to the identity of the rabbit model of atherosclerosis with atherosclerosis under clinical conditions.

It can be supposed that in our experiments just as in the experiments of E. I. Chazov rabbits with experimental atherosclerosis have disturbances in the anticoagulative mechanisms which are possibly aggravated during an acute stimulation of the nervous system. These general conditions, taken in combination with the neuro-paretic state of the cardiovascular system, are responsible for the appearance of predisposition to coronary thrombosis.

The interpretation of the mechanism of coronary thrombosis in experimental atherosclerosis and disturbance of the cardiac innervation corresponds to I. V. Davydovsky view of the decisive role played by acute spastic and paretic phenomena in the heart vessels during thrombus formation. The possibility of thrombus formation in the heart vessels as a result of a reflex disturbance in the vascular system of the heart is also pointed out by S. S. Weil.

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IMMUNOLOGICAL PROPHYLAXIS OF EXPERIMENTAL ATHEROSCLEROSIS

M. N. SULTANOV

During the past few decades, in addition to a more elaborate study of the pathogenesis of atherosclerosis, certain measures for its prophylaxis and therapy have been developed (A. L. Myasnikov).

The role of low-density lipoproteins in the development of atherosclerosis was established after the extraction of individual protein fractions from the blood plasma by means of an ultracentrifuge (Gofman et al.) and a study of their physicochemical properties (Cohn et al., Gofman; Turner et al.; Havel). There was found a definite correlation between the cholesterol content of two principal lipoproteins (α and β) and the clinical symptoms of atherosclerosis (Russ et al.), a close relationship between cholesterol and low-density lipoproteins (Gofman), a high cholesterol level, particularly in β-lipoproteins (Page et al.), the cholesterol concentration in atherosclerosis increasing by another 20 per cent (Oliver et al.). Finally, this was confirmed by the discovery of a higher concentration of β-lipoproteins in the early stages of atheromatous plaques (Tracy et al.). It was found at the same time that the deposition of lipids in the vessel wall had been preceded by deposition of β-lipoproteins within it (Gofman et al.; Gerö et al.). These data form the basis for the current view that the increased cholesterol content of the blood in the presence of changes characteristic of atherosclerosis retains its importance as an index of the development of atherosclerosis but an increase in the concentration of low-density lipoproteins better reflects the state of the process.

It has been found as a result of biochemical as well as histo-

chemical studies that in atherosclerosis the deposition of fatty substances in the aorta both under clinical and experimental conditions is preceded by an increase of a metachromatically stained mucoid constituent within aortic tissue. For example, Gerö and his collaborators have proved histochemically that the deposition of lipids takes place mainly in those portions of the aorta which are rich in metachromatically stained substances. Subsequently, upon separation of mucopolysaccharides into two fractions it became possible to establish that a fraction possessing a lower motility, showing a positive reaction to Schiff's reagent, pure human lipoprotein or fresh human plasma causes the migration of lipoproteins. This points to the formation of specific complexes between mucopolysaccharides and β-lipoprotein.

The significance of the disturbance of the mucopolysaccharide content in the genesis of atherosclerosis has been emphasized by Wartman, Schwartz and their co-authors who observed depolarization of the mucopolysaccharides of the principal constituent of the arterial wall during the development of an atheroma in cholesterol-fed rabbits. At the same time, a reduction in the lipolytic activity of the aortic tissue was also noted (Gore a. Larkey) (quoted from S. M. Leites and Chou Su).

The subintimal accumulation of mucoid substances in the early stages of atherosclerosis in many species of animals has been found by Lindsay et al., Bollet et al., Taylor, Faber, Altschuller et al. Consequently, lipid depositions may be caused by certain specific changes in mucopolysaccharides and the mucoid structure of the vessel wall.

Thus, the deposition of lipids in the vessel wall occurs mainly with the participation of β-lipoproteins, which is preceded by a change in the metachromatically stained mucoid substance of the principal constituent of the vessel wall with a decrease in its lipolytic activity.

Without going into the details of the importance and efficacy of the numerous therapeutic remedies currently used for atherosclerosis and the variable food rations it can be noted that at best they are capable of reducing hypercholesterolaemia and preventing to a certain extent the increase of lipoidosis, but fail to bring about a reversal of the existing atherosclerotic changes.

Therefore, during the past few years, investigations have been carried out in search of the most reliable and rational method for atherosclerosis prevention.

During the separation of protein fractions by means of an ultracentrifuge, Gofman and his collaborators discovered in the blood serum of atherosclerotic subjects the so-called pathological con-

centrations of lipoproteins which they attributed to the development of atherosclerosis in man and animals.

As a result of the study of these pathological lipoproteins, Kunkel, Gitlin and others discovered immunochemical heterogeneity of β-lipoproteins of the plasma while Baker and his collaborators succeeded in producing an immune serum by immunization of rabbits with the lipoprotein fraction of the blood of dogs kept on an atherogenous diet.

Subsequently, this highly interesting immunological aspect of atherosclerosis increasingly attracted the attention of researchers (Morison, Korngold et al., Levine et al.). The results of their studies confirm the antigenic property of lipoproteins not only in animals but also in human beings with generalized and coronary atherosclerosis. It has been found, for example, that in cholesterol-fed animals macromolecular antigens are produced which are very much like the antigens observed in the blood serum of patients with atherosclerosis.Another characteristic feature is the fact that these antigens are not found in healthy persons.

Using the precipitation test, Morison and others succeeded in finding out the immunological differences between the serum lipoproteins of patients with coronary thrombosis and myocardial infarction on the one hand and analogous lipoproteins obtained from healthy persons on the other hand. It also proved possible to establish not only the heterogeneity of serum β-lipoproteins (Gitlin), the character of the antigen-antibody complex but also to establish that certain parts of lipids in the atheromatous intima are contained in the form of lipoproteins of plasmatic origin (Otto; Kayden et al.).

Gerö and his collaborators (1961) during a postmortem study of homogenates obtained from the pulmonary artery, the inferior vena cava, and the aorta of atherosclerotic persons established that the precipitate to β-lipoproteins is discoverable only in aortic homogenates. It is characteristic that in none of the homogenates was the precipitate to α-lipoprotein discovered.

Taking into consideration all the above-mentioned properties of β-lipoproteins, particularly their capacity to form the antigen-antibody complex, Gerö and his collaborators (1959-1961) for the first time carried out the immunization of animals with a β-lipoprotein fraction isolated from the hypercholesterolaemic animal serum in order to find out whether the atherosclerotic process is thus retarded. The investigations yielded positive results. In cholesterol-fed animals following preliminary immunization with β-lipoproteins atherosclerotic changes were either absent or comparatively unmarked. The same authors established that the human γ-globulin, tetanus anatoxin and other antigens lack these properties.

Considering the importance and urgency of this problem, in 1961 we embarked on the study of immunological prophylaxis of experimental atherosclerosis at the suggestion of Prof. A. L. Myasnikov.

Following induction of experimental atherosclerosis in cockerels and rabbits (rabbits were given 1 g and cockerels 2 g of cholesterol daily during a period of 100 days as an admixture to maize brain without adding oil), the animals were killed by bleeding. The hypercholesterolaemic serum thereby obtained was ultracentrifugated after Gofman's method (1950) in Czuppon's modification so as to recover the β-fraction of lipoproteins, which was identified by means of paper electrophoresis. The recovered fraction of β-lipoproteins was used for cross-injection in a certain admixture of sodium chloride, and phenol solutions and aluminium hydroxide gel to healthy animals, i.e., rabbit β-lipoprotein was injected to cockerels and cockerel β-lipoprotein was injected to rabbits. The immunization was conducted once a week until the titre of antibodies rose to 1 : 10,000 and higher, which was achieved after six injections. Then the animals were kept on a cholesterol diet during 100 days after the above-mentioned method. The character of the antibodies produced was determined by means of immunoelectrophoresis which revealed the formation in the animals' blood of specific anti-β-lipoprotein antibodies (see photoimmunoelectrophoregrams).

The experiments were carried out on eight Chinchilla rabbits weighing 2.5-3 kg and eight full-grown Leghorn cockerels weighing 1.2-1.5 kg which had been preliminarily cross-immunized with a β-lipoprotein fraction. The control group consisted of six rabbits and seven cockerels of the same breeds and weight which had been fed with cholesterol during 100 days without immunization.

During the experiments all the animals were kept under identical conditions. The experiments were started after an obligatory 10-14 days' period required for the animal body to become adapted to new surroundings and food, which, we believe, is especially important for animals expected to be immunized. An indispensable pre-requisite for a successful immunization was obligatory control over the state of the titre of antibodies in the period of cholesterol feeding. In case the titre was reduced an additional immunization was undertaken. Besides, for a constant maintenance of the titre level in the period of cholesterol feeding the animals were twice subjected to supporting immunization.

In the blood of experimental and control animals before cholesterol feeding, in the period of immunization (once in every two weeks) and through the entire term of cholesterol feeding (once in every three weeks), the cholesterol level was determined by Engelgardt-Smirnova's method and that of mucopolysaccharides as follows: sialic acid—after Svenerholm and protein-bound hexoses—after Weimer and Moschin. Upon completion of the experiments the animals were killed by bleeding. The aorta was extracted whole—from the semilunar valves to the bifurcation whereupon it was fixed in formaldehyde solution and stained with Sudan III.

In the period of immunization, the cholesterol content of the blood, as is seen in Fig. 1 is slightly increased: in rabbits by 19.5 mg per cent, in cockerels by 35.1 mg per cent on the average.

In the period of cholesterol feeding both β-lipoprotein-immunized rabbits and cockerels and cholesterol-fed, non-immunized

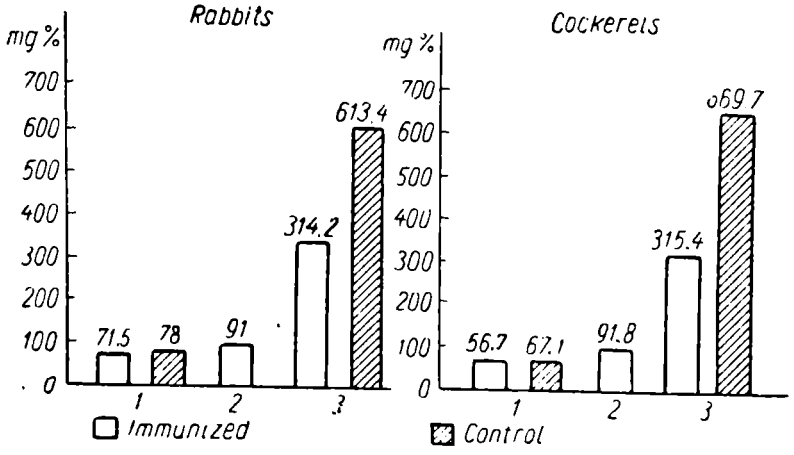

Fig. 1. Changes in the cholesterol content of the blood in β-lipoprotein-immunized and control rabbits and cockerels.

1—before experiment; *2*—during immunization; *3*—during cholesterol feeding

(control) animals were found to have an increased cholesterol concentration in the blood. The cholesterol content of the blood in immunized animals was lower than in the control group by an average of 48.8 per cent and 52.9 per cent in rabbits and cockerels respectively.

It was found by means of biochemical, as well as histochemical investigations that the change in the structure of mucopolysaccharides of the vessel wall during the development of atherosclerosis coincided with the changes in the mucopolysaccharide content of the blood plasma. For example, in patients with atherosclerosis one can note an extremely marked increase in the concentration of hexoses and mucoproteins as well as a rise of the hexosamine and sialic acid level (Gerö et al.; Tracy et al.).

The studies we have carried out in determining the mucopolysaccharide content of the animal serum have shown that the level of protein-bound hexoses, as is seen in Fig. 2, in animals preliminarily immunized with β-lipoproteins and then fed with cholesterol increased on the average by 44.5 γ in rabbits and 22.7 γ in cockerels, whereas in the control animals the increase was more pronounced: rabbits 78.0 and cockerels 48.0 γ on the average.

The greatest interest was evoked by a change in the level of sialic acid—an acetylated product of neuraminic acid. For example, in β-lipoprotein-immunized animals, both in the period of immunization and during cholesterol feeding, the sialic acid content of the blood serum remained unchanged or tended to reduce, particularly in the period of immunization, whereas

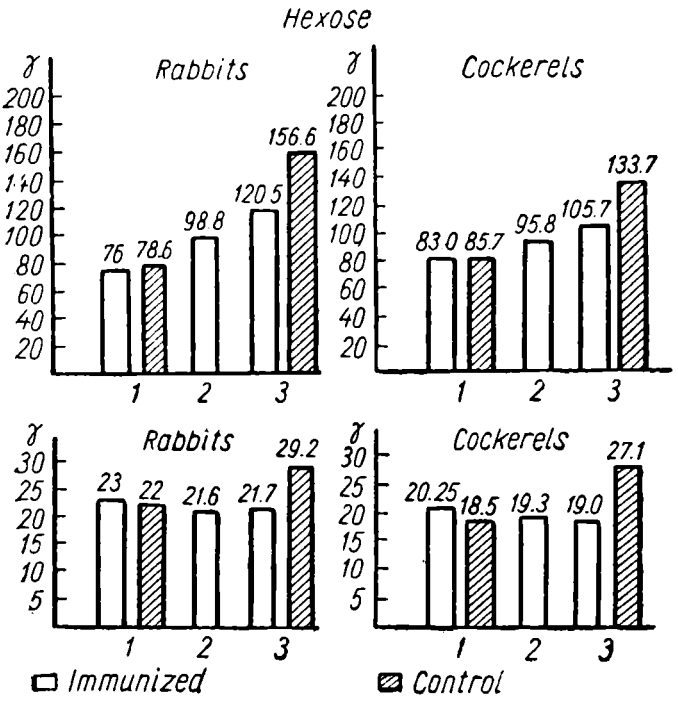

Fig. 2. Change in the level of protein-bound hexoses and sialic acid in the blood serum of β-lipoprotein-immunized and control rabbits and cockerels. Same designations as in Fig. 1

in the control animals in the period of cholesterol feeding, as is seen in Fig. 3, its concentration increased by 7.2 γ in rabbits and by 8.6 γ in cockerels on the average. These data agree with those furnished by Gerö and his collaborators.

It should be noted that neuraminic acid and its derivative, sialic acid, are normal constituents of the blood, the cerebrospinal fluid and other organs and fluids of the body and are of great biological importance. Changes in their levels are noted in various pathological states.

It is interesting as well as highly important to know that neuraminic acid takes part in the defence systems of the body (V. I. Tovarnitsky), while Cenciotti (cited from I. V. Tsvetkova) holds that a decrease in its content may be accounted for by the participation of neuraminic acid in the development of immunity. The role of neuraminic acid in the production of the immunity mechanism is noted by Michel et al. (cited from I. V. Tsvetkova).

In view of the above-mentioned specific properties of neuraminic acid, it can be presumed that a decrease in the level of sialic acid in the animals we had immunized with β-lipoproteins, particularly in the period of immunization, was associated with its role in the production of immunity.

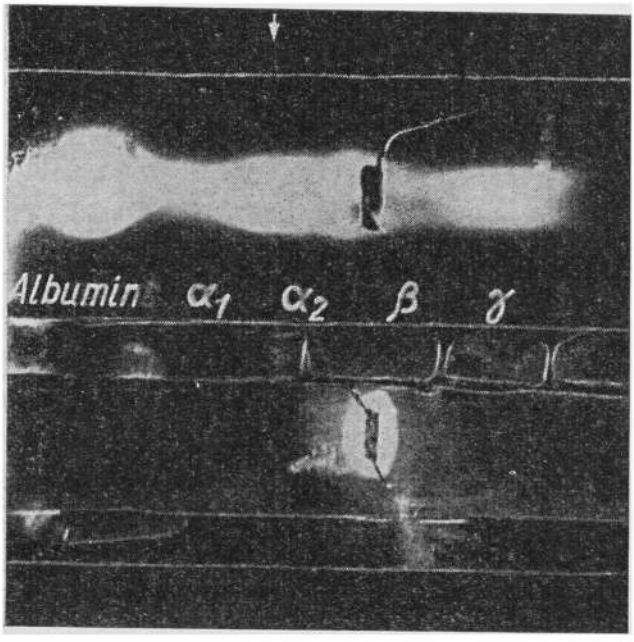

Fig. 3. Immunoelectrophoregram. Upper picture shows distribution of immune serum according to protein fractions, lower picture—the formation of specific precipitative anti-β-lipoprotein ring

Statistical processing proved the significance of these data (see table).

The results of morphological investigation of the animals' aorta were as follows: of the 16 preliminarily immunized cockerels and rabbits the development of atherosclerosis of the 1st degree was noted in two cockerels and of the 2nd degree in one cockerel. In the remaining five cockerels and in all eight rabbits only isolated deposits of lipoid infiltration or no changes at all were discovered in the aorta. All of the 13 control animals were found to develop atherosclerosis of the II-III degree. The better effect of retarding the atherosclerotic process in β-lipoprotein-immunized animals as compared to the findings of Gerö and his collaborators is, in our opinion, due to a certain alteration in the method of immunization owing to the use of a specially prepared aluminium hydroxide gel which apparently provides a more reliable depot for a prolonged and uniform maintenance of immunity.

Preliminary cross-immunization of cockerels and rabbits with β-lipoproteins, as is evidenced by the above-given data, exerts a favourable specific influence and brings into play factors not only contributing to inhibition of hypercholesterolaemia but also to certain other changes on which the development of athero-

RESULTS OF STATISTICAL PROCESSING OF DATA FROM DETERMINATION OF BLOOD CHOLESTEROL AND BLOOD SERUM MUCOPOLYSACCHARIDES

	Cholesterol content, mg/per cent		Protein-bound hexoses content, γ		Sialic acid content, γ	
	rabbits	cockerels	rabbits	cockerels	rabbits	cockerels
Initial data	71.5±6.95	56.7±6.74	76.0±11.55	83.0±10.40	20.0±1.573	20.25±1.12
β-lipoprotein immunization field	91 ±5.51	91.8±7.51	98.8±6.26	95.8±5.8	21.6±1.889	19.3±1.156
Immunization + cholesterol	314.2±52.09	315.4±48.5	120.5±10.99	105.7±5.59	21.7±1.79	19.0±0.855
Cholesterol without immunization	613.4±218.07	669.7±174.99	156.6±21.4	133.7±7.26	29.2±2.09	27.1±1.85

sclerosis depends. Another evidence in favour of this is the fact that in certain immunized animals, in spite of an increased cholesterol concentration in the blood (sometimes to 490 mg per cent), accompanied by definite changes in the mucopolysaccharide content, no development of atherosclerosis was noted.

The mechanism of the inhibitory influence of immunization with β-lipoproteins on experimental atherosclerosis is accounted for, as is noted by Gerö and his co-authors, by the formation of a complex combination of β-lipoproteins with atherogenous macromolecular lipoproteins whose level rises as a result of keeping the animals on a cholesterol diet. At the same time, it is connected with a change in the mucoid structure of the principal constituent of the vessel wall as a result of the reciprocal antigen-antibody reaction which takes place in the principal constituent of the connective tissue. It should be supposed, in addition, that following preliminary immunization during feeding the animals with cholesterol the administered β-lipoproteins contribute to an earlier formation of macromolecular lipoproteins. This is what to a certain extent prevents further changes associated with cholesterol feeding.

If at the onset of atherosclerosis β-lipoproteins are some of the indispensable parts of the complex factors responsible for the development of atherosclerosis, in the process of its aggravation they acquire an antigenic property and probably interfere with the progression of atherosclerosis. This may be due to the properties of β-lipoproteins, which differ from other lipoproteins for their lability and decreased resistance to denaturation agents (Gottfried et al.).

It can be presumed that similar adaptational changes take place in the human body since an early age in case of disturbance of the protein-lipid complex or deposition of lipids in the vessel wall. Apparently, at the same time, lipoidosis may show a reversal depending on the intensity of the adaptational defense reaction of the body, whereas in the absence of this capacity atherosclerotic changes may develop.

We also made efforts to find out how long a cholesterol diet should be for the above changes to happen. In the experiments the serological method of precipitation on agar was used as well as immunoelectrophoretic study. On the 36th day of keeping the animals on a cholesterol diet we succeeded in discovering β-lipoproteins possessing an antigenic property. Further studies in this direction are required, however.

The results of our experimental research indicate that preliminary immunization of animals with β-lipoproteins exerts an inhibitory influence on the development of experimental atherosclerosis. This influence is apparently due to the elimination

of factors required for the development of atherosclerosis both on the part of the metachromatically stained mucoid substance of the principal constituent of the vessel wall and on the part of the protein-lipoid complex and the complicated biochemical processes associated with it.

CONCLUSIONS

1. Preliminary immunization of animals with β-lipoproteins recovered from hypercholesterolaemic serum produces an inhibitory effect on experimental atherosclerosis.
2. The inhibitory effect of preliminary immunization with β-lipoproteins on experimental atherosclerosis is apparently due to incipient changes in the principal constituent of the vessel wall as well as the protein-lipid complexes of the blood serum.
3. During the development of atherosclerosis, a certain part of β-lipoproteins apparently acquires antigenic properties, which is a manifestation of the defence adaptational reactions of the body interfering to a certain extent with the further progression of atherosclerosis.

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AN ELECTRON MICROSCOPIC STUDY OF THE CORONARY ARTERIES OF THE RABBIT HEART IN CHOLESTEROL ATHEROSCLEROSIS

A. I. KLEMBOVSKY

The purpose of the present research was to reveal in experimental cholesterol atherosclerosis the earliest changes in the wall of the coronary arteries which occur before the accumulation of lipids and are discoverable by electron microscopy; simultaneously the vessels were also studied by means of light microscopy.

The object of study was the left coronary artery in ten rabbits which were given cholesterol in a dose of 0.25 g per kg of body weight. The rabbits were slaughtered within a period of 1 to 40 days after the beginning of the experiment. The same vessels were studied in five control rabbits not fed with cholesterol. The tissue under study was subdivided into two groups according to the method used for making the preparations for light or electron microscopy; the tissue of each group was separately treated and studied. The material was fixed in neutral isotonic formaldehyde solution or in osmium tetroxide, put into paraffin, gelatin or a mixture of methacrylates, treated by means of the conventional histological and certain histochemical methods (for lipids, acid mucopolysaccharides, fibrin and elastic tissue). The preparations for electron microscopy were obtained using the method of ultrathin sections. They were examined under ЭМ-3 and УЭМБ-100 electron microscopes obtaining up to 25,000 times magnification on the screen.

The results of the investigation indicate that the incipient changes in the structure of the coronary arteries take place rather early and are noted in all the three layers of the vessel walls. In the proximal portion of the arteries they are considerably brighter, in the distal ones they are paler and less permanent. The earliest changes in the vessel wall are seen, as a rule, in the in-

tima; in certain animals, however, the first changes in the structural elements were noted in the media and even in the adventitia.

The earliest changes in the vessel structure were discovered by means of electron microscopy on the 2nd day (in two animals) and on the 4th day of the experiment.

Our findings are presented in microphotographs (Fig. 1*A*, *B*, *C*, and *D*, and Fig. 2).

The intima of the coronary arteries in control animals kept on a cholesterol-free diet showed a comparatively even contour of the endothelial cells, the presence of a subendothelial layer between the lower border of the cells and the inner elastic membrane, and, in addition, distinct connections between the elastic membrane and the smooth muscle cells of the media (Fig. 1*A*).

On the 3rd-4th day of the experiment the endothelial cells of the rabbit artery showed a marked increase, as compared to the control, in the tortuousness of the cell membrane and nucleus, the mitochondria and the elements of the endoplasmatic network being more clearly seen, and the cell membrane showing deeply indrawn parts almost reaching the cell nuclei.

These morphologic signs, according to Buck and other authors (Kish), point to an increased activity of the endothelial cell in lipoprotein metabolism. This is possibly a manifestation of the "physiological reaction" of hypercholesterolaemia by the arterial wall.

Rabbits slaughtered on the 4th, 6th and 8th day of cholesterol feeding showed more striking changes in the vessel structure related apparently to the field of pathology, namely, the osmiophilic deposits on the free surface of the endothelial cells (Fig. 1*B*). In addition to the deposition proper there is vacuolization of the nucleus of the cell carrying osmiophilic tissue, as well as signs of penetration of the cell by small portions of osmiophilic substance by way of "pinocytosis" (Palade, Moore and Ruska). In the region of the osmiophilic caps the subendothelial layer is absent or inadequate.

We believe that focal osmiophilia manifests injury to the cell surface on which the lipoprotein complex falling out of the plasma is localized. Possibly, it contains fibrin or fibrinogen, as is supposed by Duguid and Parker. It is known that electron microscopy reveals fibrin fibers in atherosclerotic plaques (Levene).

In the study of preparations, one's attention is drawn to a characteristic change in elastic fibres, which may be incipient among all the other changes in the wall structure of the coronary artery. One can see, in particular, focal thickenings of the internal elastic membrane of the rabbit artery 6-8 days after the experiment. The endothelial cells are at the same time displaced towards

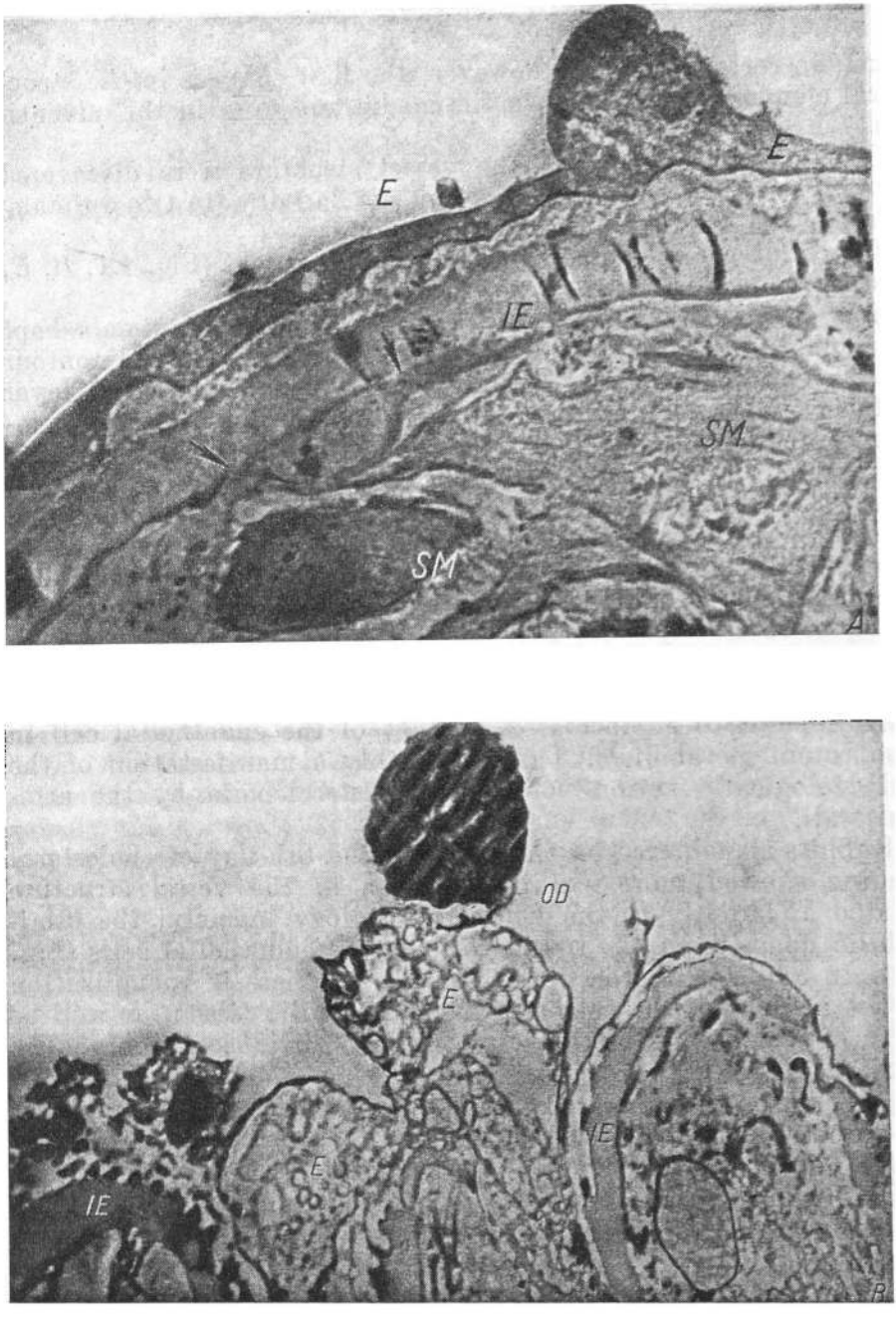
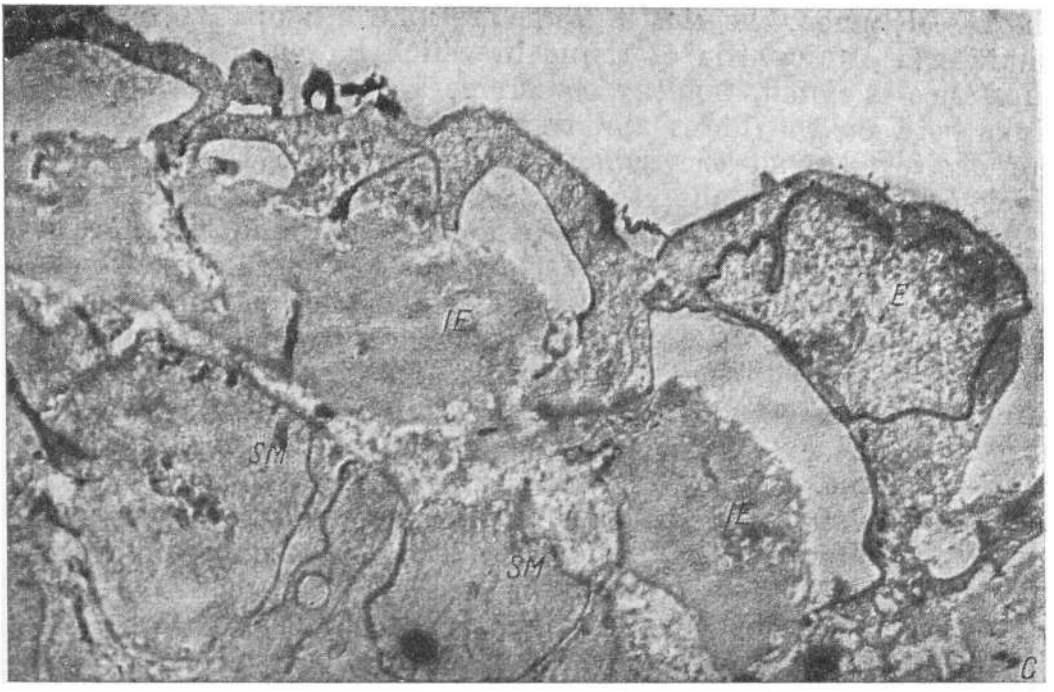


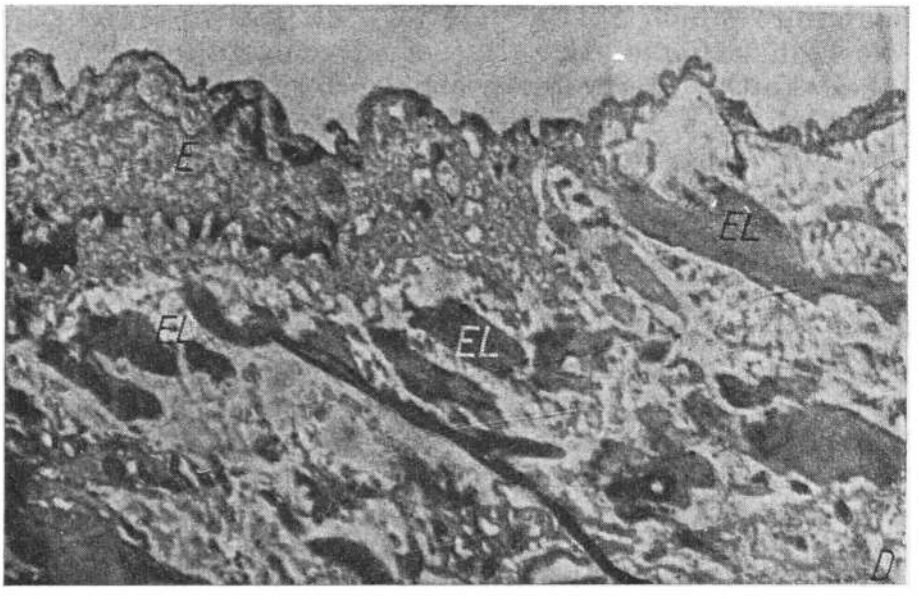
Fig. 1. Electron microphotographs

Walls of the coronary arteries in rabbits kept on a cholesterol diet: *E*—cytoplasm and endothelial nuclei; *IE*—internal elastic membrane; *SM*—smooth muscle cells of the media.

A—artery of rabbit not kept on a cholesterol diet; *IE*—of approximately equal thickness all over its length; *SM*—connected with *IE* (indicated by arrows). Magnification 7,300 times

B—osmiophilic deposition (*OD*) on free surface of endothelial cell (48 hours of experiment). Magnification 15,000 times





C—focal swelling of *IE* accompanied by loosening of elastic tissue and disorganization of links between fibres and *SM* (8 days of experiment). Magnification 15,000 times

D—disintegration of *IE* with conversion of fibres into lumps of elastic tissue (*EL*). Tortuous contours of the cytoplasm and the endothelial nuclei are visible (3 days of experiment). Magnification 7,300 times

the vessel lumen, the elastic fibres are uneven and diverse due to ununiform osmiophilia of tissue in which loosening of the fibre substance is noted, lending the fibres a porous appearance; the links between the fibres and the adjoining smooth muscle cells are destroyed, and the subendothelial layer in the section of thickened fibres is absent (Fig. 1*C*).

A second variety of changes in the state of the elastic fibres is manifested by fragments of elastic fibres in the thickened oedematous osmiophobic layers of the artery (the intima and the media). As a rule, the internal elastic membrane of the vessel is absent, and there are signs of an increased passage of the substance through the endothelial cell, which is manifested by the tortuousness of the external membrane of the cytoplasm and by expansion of the network of endoplasmatic spaces as well as by the irregular shape of the endothelial nuclei. A similar phenomenon was noted as early as 48 hours after the experiment and later (Fig. 1*D*).

In this connection, we recall studies on atherosclerosis by Bredt, Lindner and other authors who noted oedema of the arterial intima in man and in an experiment during light microscopy.

The media of the coronary artery in the rabbit on the 6th-8th day of the experiment and later showed thickenings of the elastic membranes which in the majority of cases were combined with disorganization of the sites of attachment of the smooth muscle cells to the elastic fibres, attended with vacuolization and increase of tissue osmiophilia in these sites.

In *the adventitia*, on the 6th-8th day of the experiment and later, the number of vacuolized cells containing lipoproteins increased and the elastic fibres thickened also. In three rabbits out of ten thickened elastic fibres of the aortic adventitia were the first visible changes in the vessel-wall structure.

The first inclusions of lipids were noted by the method of electron microscopy on the 6th-8th day of the experiment. They were simultaneously observed both in the endothelial and the smooth muscle cells as well as outside of the cells, near the elastic fibres.

At the same time, light microscopy of the arteries of animals kept on a cholesterol diet during not more than 10 days, revealed no lipids in preparations stained after Goldman. In such objects also, however, a number of structural changes could be noted: irregular thickening, blurred contours (seen as an "excrescence") and increased tortuousness of the internal elastic membrane and the elastic fibres of the media; ruptures of the internal elastic membrane; insignificant microhaemorrhages in the intima of the arteries; the formation of focal thickenings in the intima shaped as "cushions" covered by the endothelium or without it, which

might be combined with swelling and breaking up of the underlying elastic fibres.

A parallel study of the same arteries by means of electron microscopy has proved that such changes in the vessel structure are not artefacts. Indeed, in spite of the difference in fixation, staining and microscopy of the coronary arteries of the heart in rabbits they show similar changes when studied by light or electron mieroscopy.

The first signs of arterial lipoidosis, according to data of light microscopy, appear in rabbits after a ten-day cholesterol diet. They consist of small areas of dust-like accumulations of lipids in the intima arranged mostly along the course of the elastic fibres and, in addition, accumulations of small lipid drops in the cushion-shaped swellings of the intima.

Subsequently, in experiments lasting from 15-20 to 30-40 days atherosclerotic changes in the coronary arteries occurred in two principal forms: firstly, as xanthomatous plaques, sometimes very small, protruding into the vessel lumen, and secondly, as areas of lipoidosis of the intima and partly the media of the artery lying outside of the plaques and unaccompanied by considerable changes in the structure of the arterial wall elements. At a later time of the experiment (30-40 days) it becomes difficult to delimit both types of changes.

Acid mucopolysaccharides detected by aid of histochemical reactions are often discovered in a considerable amount both in the intima and the media of the vessels in experimental animals. In earlier periods of the experiment, however, as well as in the period of appearance and accumulation of lipids no increase in mucopolysaccharides is noted as compared to the control cases. This agrees with the findings of many authors who point out that the accumulation of mucopolysaccharides takes place at comparatively late stages of atherosclerosis.

The aggregate of changes in the coronary arteries under cholesterol load detected in electron microscopic preparations in periods of experiment lasting up to 40 days is as follows: (1) tortuousness of the endothelial cell contours and widening of the spaces of the endoplasmatic network; (2) osmiophilic depositions on the free surfaces of endothelial cells; (3) irregular change of the thickness and osmiophilia of the internal elastic membrane and lumped disintegration of elastin in different layers of arteries; (4) destruction of connections between the elastic fibres and the smooth muscle cells and isolation of the latter; increase of the light space between the changed fibres and cells; (5) appearance of osmiophilic and vacuolized lipid inclusions in the endothelium and the smooth muscle cells of the media, as well as of extracellular osmiophilic inclusions (Fig. 2).

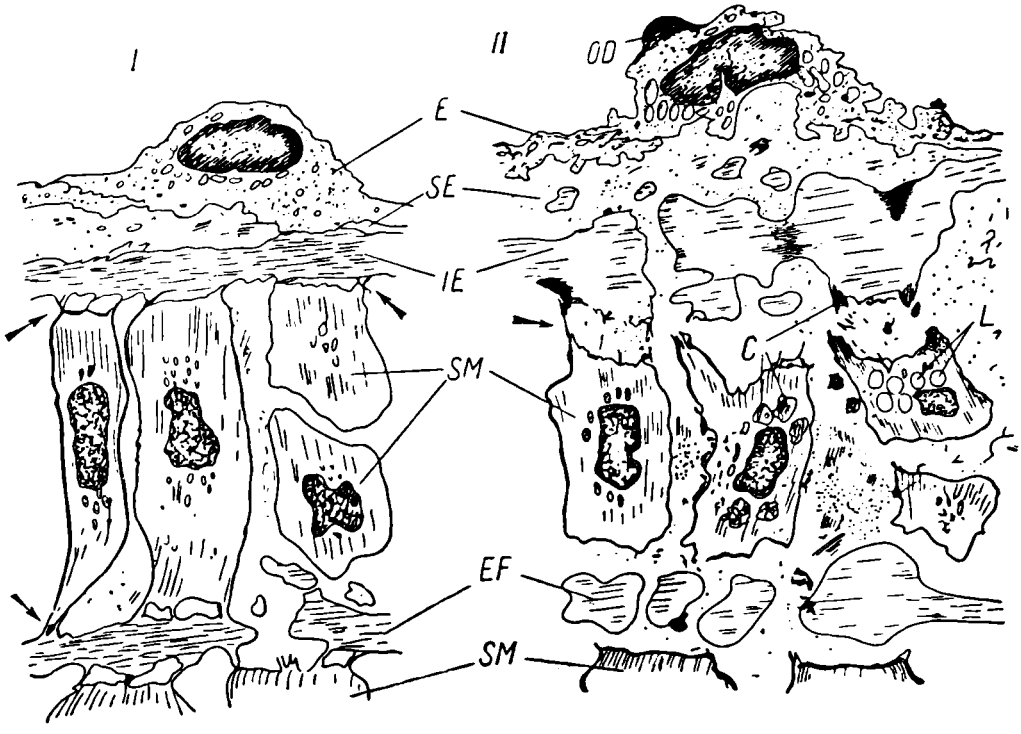


Fig. 2. Scheme of fine structural changes in the coronary artery of a rabbit kept on a cholesterol diet, drawn up from electron microphotographs.

I—intact artery of control animal; *II*—artery of experimental animal; *E*—endothelium; *SL*—subendothelial layer; *IE*—internal elastic membrane; *SM*—smooth muscle cells of the media connected with elastic fibres (indicated by arrows); *L*—lipid inclusion bodies; *OD*—osmiophilic deposit; *C*—collagen fibres; *EF*—elastic fibres

The results of the observations show very early morphological changes in the coronary arteries of the heart in experimental animals. In 24-28 hours after the beginning of the experiment the endothelial cells are noted to take an active part in lipoprotein metabolism; osmiophilic depositions appear on certain cells of the endothelium, and oedema of the intima and media is noted. In 2-3 days (sometimes earlier) the structure of the elastic tissue in the arteries of the rabbit heart is changed, which is the easiest to notice in the internal elastic membrane of the vessels. This sign may be the first among all other changes in the arterial wall. Almost simultaneously (on the 3rd-4th day of experiment) swelling and reduction of osmiophilia of the elastic fibres in the media of the rabbit vessels are noted; such a change in the elastic fibres is sometimes observed in the adventitia of the rabbit arteries. Lipid inclusions are noted later, on the 6th-8th day of the experiment.

A much similar change has been reported to occur in the rabbit aorta under the same experimental conditions and methods of study (A. F. Ushkalov, A. I. Klembovsky).

The above findings are a good reason for doubt about lipoidosis being invariably the first sign of experimental atherosclerosis (N. N. Anichkov).

There is a view that atherosclerosis, including experimental atherosclerosis in rabbits, is associated not only with disturbances in lipocholesterol metabolism (A. L. Myasnikov). Proceeding from our own findings we can presume that the initial sign of experimental atherosclerosis may be a change in the elastic tissue manifesting a disturbance in protein metabolism. Indeed, in the arteries of animals with induced atherosclerosis as early as on the 6th day of the experiment even light microscopy revealed dystrophic and destructive changes in the elastic fibres of the coronary arteries or oedematous thickenings in the intima with damage to the endothelial covering and microhaemorrhages in the intima. As is shown by electron microscopy, they are not artefacts. In addition, the same kind of changes in the elastic fibres as the earliest signs of atherosclerosis is pointed out by a number of authors. For example, the Hungarian authors Balo and Banga maintain that a primary change of elastic fibres is possible in atherosclerosis.

As a working hypothesis, the onset of experimental cholesterol coronary atherosclerosis in rabbit may be described as follows.

Under the influence of keeping the animals on a cholesterol diet dystrophic processes occur in the elastic fibres of the intima of the arteries attended with disturbance of the permeability of the vessel wall. These processes are accompanied by changes in the properties of the endothelial covering, destructive changes in the elements of the intima and oedema of the intima and microhaemorrhages. It is possible that osmiophilic depositions on the endothelium should be regarded as a sign pointing to a local, probably primary, disturbance in the structure of the internal surface of the arterial wall, where an atherosclerotic plaque may be formed.

Later, cushion-shaped thickenings which are microfocal plasmorrhages into the intima appear on the structurally changed portions of the arterial walls. After a time, lipids appear in these thickenings. Then, apparently as a result of the activity of subendothelial macrophages the thickenings turn into xanthomatous plaques. The primary changes in the elastic fibres may possibly be a localizing factor in the formation of a plaque in cholesterol atherosclerosis.

Nearly all of the findings obtained may be explained from the viewpoint of the infiltrative theory of pathogenesis of experimental atherosclerosis. The initial changes in the elastic tissue and lipid accumulations, however, are observed not only in the intima but also in the media or adventitia. This phenomenon

makes one ponder over the significance of the vasa vasorum in the development of atherosclerosis or synthesis of lipids by the elements of the arterial wall itself, since infiltration of lipoproteins from the vessel lumen under study is not apparently responsible for the above-mentioned facts.

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MORPHOLOGIC CHANGES IN THE AORTA OF MONKEYS WITH EXPERIMENTAL ATHEROSCLEROSIS

A. F. USHKALOV, A. I. KLEMBOVSKY

The solution of certain problems relating to experimental cholesterol atherosclerosis in monkeys is of considerable interest in view of the similarity between the biological systems of the monkey and man.

The production of an experimental model of atherosclerosis by addition of cholesterol to the food of monkeys is complicated by a number of difficulties. For example, Hueper failed to induce atherosclerotic changes in vessels in monkeys kept on a cholesterol diet, whereas Mann and his collaborators and Taylor et al. succeeded in provoking such changes.

E. I. Chazov of the Institute of Therapy under the USSR Academy of Medical Sciences induced atherosclerosis in monkeys through a combined influence on the animals by food cholesterol and two additional factors—pituitrin, increasing the tone of the smooth muscles of the arteries, and thrombin, enhancing the predisposition to thrombus formation.

In this experiment we have carried out the morphological part of the investigation, which is described in the present paper.

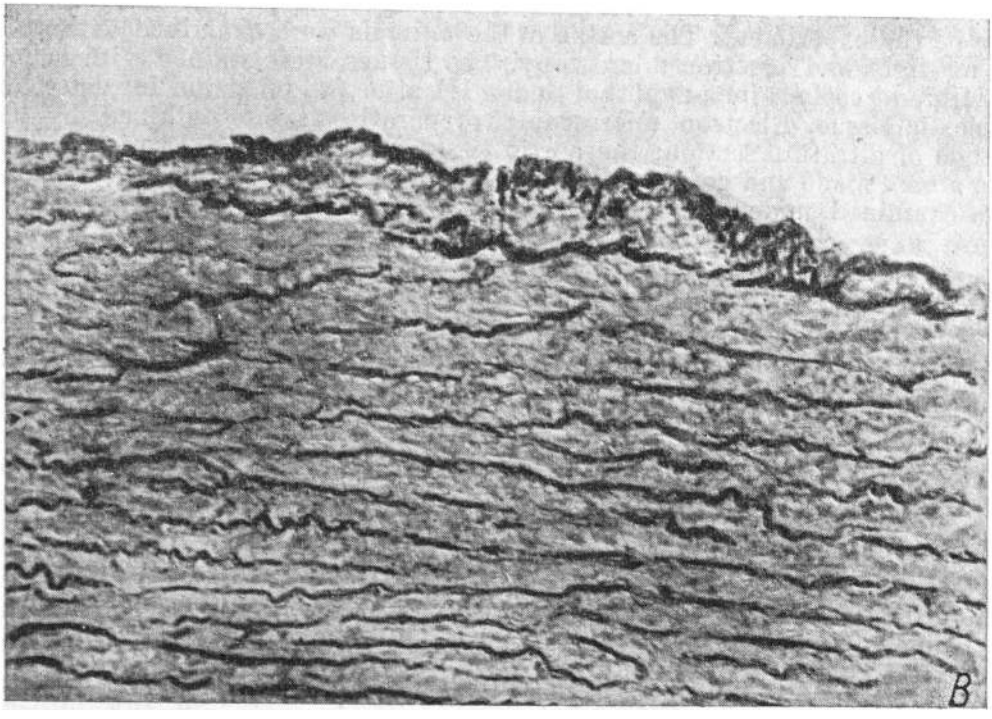
The experiments were staged on 12 Macaca rhesus monkeys weighing 4-5 kg (E.I. Chazov). Following a month long acclimatization the animals were fed with a special food mixture containing among its components cholesterol and neutral fat. In the first series of experiments six monkeys were kept on a diet with a daily cholesterol dose of 0.8-1 g per kg of body weight. In the second series of experiments six other monkeys kept on the same diet were given after nine months of feeding small doses of pituitrin and thrombin by intravenous injection during ten days (2 ml doses of thrombin solution with 12 sec activity and 0.5 ml pituitrin doses daily). All animals were slaughtered by decapitation eleven months after the begin-

Fig. 1.

A—modification of elastic fibre (*EF*) and links (indicated by arrows) between it and processes of smooth muscles (*SM*) in the media in monkeys kept for 11 months on a diet with added cholesterol and neutral fat. Picture shows an irregular osmiophily and lumped disintegration of elastic fibre. Electron microphotograph. Magnification 6,500 times

B—focal thickening of intima and homogenization of interstitial substance resembling oedema or swelling in thoracic portion of aorta in a monkey slaughtered after being kept on a cholesterol diet for 11 months and given a brief administration of thrombin and pituitrin. Stained after Goldman. Magnification 200 times

C—focal thickening of large-sized intima in aortic arch with a small number of little lipid granules (cholesterol, thrombin and pituitrin). Stained after Goldman. Magnification 200 times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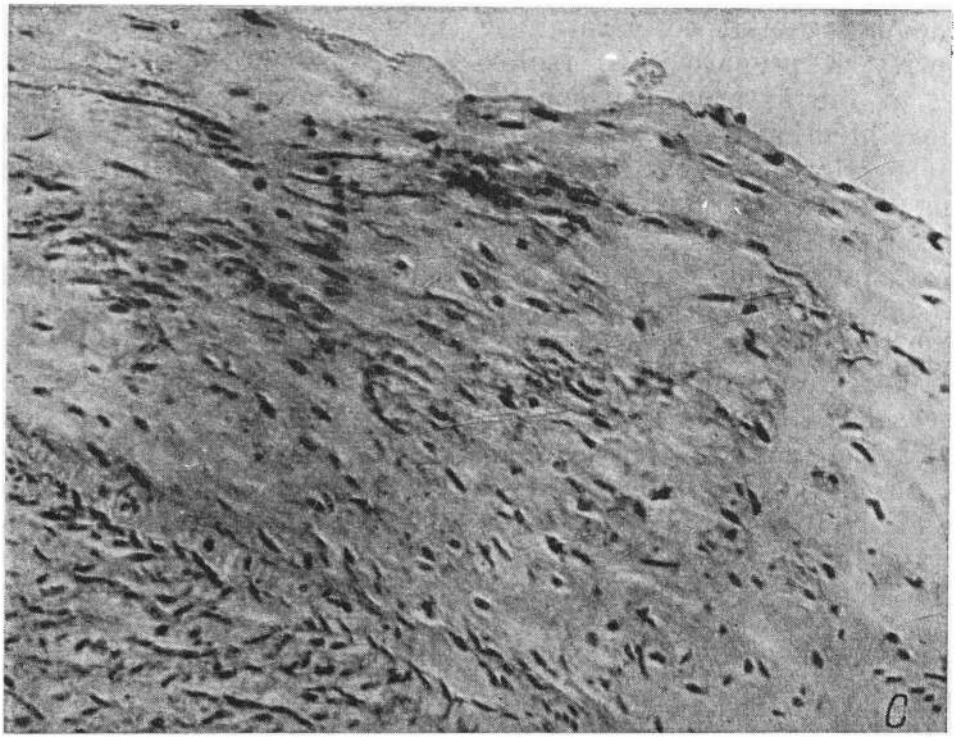
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ning of the experiment. The aortae of the animals were examined and studied by light and electron microscopy. The tissues were stained with hematoxylin and eosin, alpha-naphthol-Sudan III after Goldman and for detection of elastic tissue. Electron microscopic preparations were obtained by the method of ultrathin section; the aortic tissue was fixed with osmium tetroxide after Palade and contained in a mixture of metacrylates. The sections were examined under type ЭМ-3 and УЭМБ-100 electron microscopes.

Examination and study of the tissues of animals by light microscopy in the first series of experiments showed no changes pointing to the development of atherosclerosis. Only electron microscopy revealed in two monkeys changes which might be estimated as the initial signs of atherosclerosis.

They were focal thickenings or splitting of the internal elastic membrane and isolated elastic fibres in the media of the aorta, expansion of the spaces of the endoplasmatic network with the appearance of round or oval vacuoles in the cell cytoplasm of the smooth muscles and the connective tissue cells of the aorta. Disturbed connections were observed between the processes of the smooth muscle cells and the elastic fibres, looseness of the collagen bundles around elastin, deformation of isolated elastic fibres some of which turned into oval drop-shaped bodies seen against the widened light background formed by the principal substance of the aortic wall (Fig. 1).

The endothelial cells retained their usual appearance in the majority of preparations. Isolated sections showed rugged contours of the external surface of the endothelial cells and their nuclear membranes as well as expansion of the spaces of the endoplasmatic network filled with a slightly osmiophilic homogeneous substance, possibly lipoprotein (Buck). This substance resisted staining with alpha-naphthol-Sudan III judging by the preparations made for light microscopy. Here and there on the free surface of the aortic endothelium were noted markedly osmiophilic overlays of an optically dense substance in the form of streaks and drops. According to Duguid and Parker, the substances localized on the inner surface of the arterial wall may contain fibrin or fibrinogen. According to Parker (1960), A. F. Ushkalov and A. I. Klembovsky (1962) a similar change in the structure of the arterial wall is observed in rabbits in the early stages of experimental atherosclerosis.

The absence of marked atherosclerotic changes in animals in the first series of experiments, even those kept on a high-dosage cholesterol diet for a long time, dictates a search for additional factors so as to reproduce such structural changes which would be similar to atherosclerosis in man. At the present time, great significance in the development of atherosclerosis is attached to the anticoagulative components of the blood—heparin and

fibrinolysin. Therefore, in the second series of experiments conducted by E. I. Chazov, six monkeys in the ninth month of the experiment were given small doses of pituitrin and thrombin. Intravital laboratory examination of these animals showed that in such cases changes in the cholesterol and lipoprotein content of the blood in these animals were greater. It should be noted, however, that the increase in the cholesterol level was much less than in rabbits with induced atherosclerosis. Its level varied within a range normal for man and slightly increased for monkeys —from 180 to 225 mg per cent (normally 100-130 mg per cent). A greater change than in the first series of experiments was noted in β-lipoproteins whose level varied within a range of 67-80 per cent. A considerable reduction was also noted in the activity of the coagulative system of the body, which was manifested by a decrease in the heparin content and the fibrinolytic activity of the blood (heparin—1.8-3.7 U per ml [normally 5-6 U per ml]; fibrinolytic activity 8-12 per cent [normally 30-40 per cent]— data of E. I. Chazov).

Thus, keeping monkeys on a cholesterol diet and simultaneously influencing the coagulative system results in a marked disturbance both in fat metabolism and in the coagulative properties of the blood.

During a pathoanatomical examination of these animals changes in the aorta were discovered in four cases out of six even by a conventional observation. All of its parts were found to have portions with an uneven surface which were rather uniformly distributed. These portions protruded into the lumen as plaque-like formations of a pale-yellow or grayish colour, oval and almost round in shape or extended along the length of the vessel. Such a change of the aorta in monkeys differed from manifestations of experimental atherosclerosis in rabbits in which structural changes begin primarily in the proximal parts of the aorta.

Microscopic examination revealed significant structural changes in the aortic wall. One could note above all focal cushion-shaped thickenings of the intima with a marked dyscomplexness of the cellular elements and homogenization of the interstitial substance looking like acute oedema or swelling often extending beyond the limits of the internal elastic membrane into the inner layers of the media (Fig. 1*B*). These formations varied in size from hardly noticeable to large, reaching one third and more of the total thickness of the vessel wall (Fig. 1*C*). The majority of such thickenings contained a small amount of lipids seen as aggregations of tiny drops and grains situated outside of the cells or small areas of unmarked diffuse sudanophilia (Fig. 2). In certain thickenings, however, including those of considerable size, not even traces of lipids were discoverable (Fig. 3).

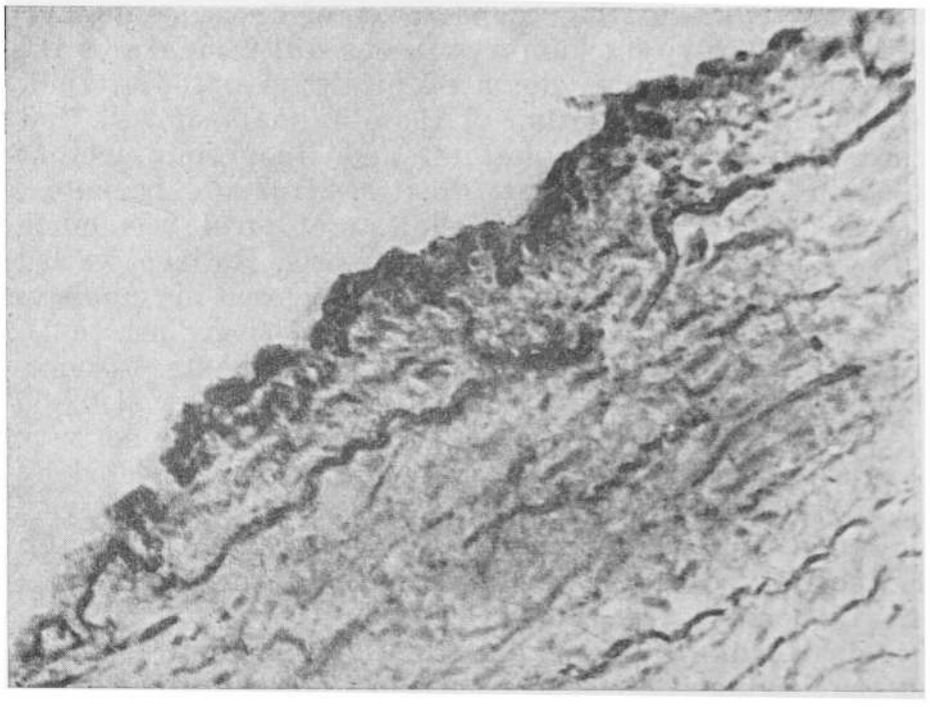

Fig. 2.

Accumulation of small drops and lipid granules in the thickened portion of the intima of the abdominal portion of the aorta; fragmentation and disintegration of the internal elastic membrane (cholesterol, thrombin and pituitrin). Stained after Goldman. Magnification 250 times

Such changes in the intima were invariably combined with marked disturbances in the elastic structures of the vessel wall, namely, the internal elastic membrane and the elastic fibres of the inner layers of the media. One could note irregularly thickened fibres with blurred contours in the form of excrescences, considerable fragmentation, areas of granular and lumped disintegration and, finally, lysis of the elastic tissue with its almost complete disappearance, sometimes over a considerable area (Figs. 2 and 3).

In addition to focal thickenings, light microscopy of the aorta in monkeys revealed slight lipoidosis in the structurally unchanged intima, mainly along the course of the internal elastic membrane, which, as is known, was noted in rabbits.

Electron microscopy of the aortic tissue in portions with focal thickenings of the intima and lipoidosis showed that the structural change in the cells and fibres of the intima and media noted earlier during examination of the aortic wall in the first series of experiments was of a much more striking character in the second series. One could note, in particular, larger irregular thicken-

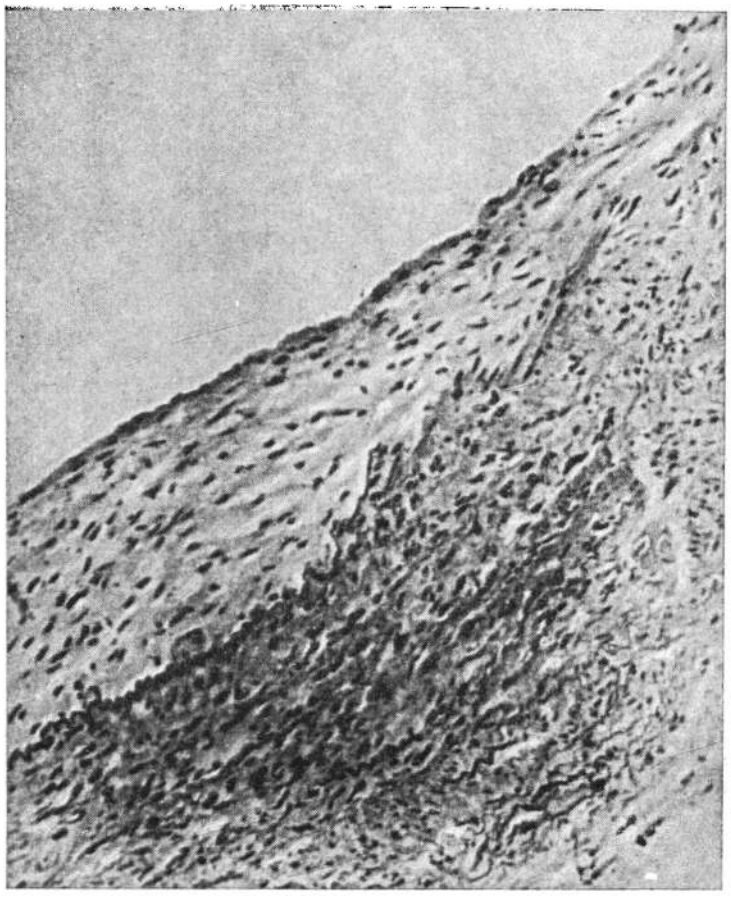

Fig. 3.

A large plaque without lipids in the abdominal portion of the aorta; disappearance of the internal elastic membrane over a considerable part of its length (cholesterol, thrombin and pituitrin). Stained after Goldman. Magnification 120 times

ings, deformation and disintegration of the elastic fibres and great disruptions in the connections of these fibres with the smooth muscle cells.

During examination of aortic preparations the endothelial cells are the first to catch the eye with their vacuolized cytoplasm in which expanded vacuoles of the endoplasmatic network of an oval and round shape are noted (Fig. 4). The density of most of these vacuoles does not differ from that of their background, and it is only in certain places of the lumina and in the walls of cavities of the endoplasmatic network that osmiophilic substance is found. These findings correspond to the electron microscopic picture of lipoidosis of the endothelial cells. As is known, fixation of tissue with osmium tetroxide does not yet guarantee preservation of lipids during tissue dehydration in ethyl alcohol when making preparations for electron microscopy (Sjöstrand). Indeed,

Fig. 4.

Endothelial cells located in a portion of an atherosclerotic plaque in the aorta of a monkey given cholesterol, thrombin and pituitrin. A relatively regular contour of the cell surface facing the vessel lumen. Expanded spaces of the endoplasmatic network (*EN*) with lipid granules (*L*). Isolated small mitochondriae (*M*). Electron microphotograph. Magnification 7,500 times

control preparations stained with Sudan show in light microscopy drops of lipids inside and outside of the endothelial cells. The membranes of the cytoplasm and nuclei of most of the endothelial cells have an oval shape and regular borders. The cell cytoplasm shows isolated mitochondriae and small osmiophilic granules of ribonucleoproteids.

This kind of cytoplasm of the endothelial cells probably points to an intracellular accumulation of lipids and reduction of cell activity in the metabolic processes occurring in the arterial wall. It should be noted in this connection that in the given phase of developing atherosclerotic changes, osmiophilic depositions are, as a rule, no longer discovered on the free surfaces of endothelial cells, as it was observed earlier in the experiment.

In animals of the second series of experiments the subendothelial layer of the aorta was considerably thickened, the structureless background occupying most of the area in sections of this layer. The fibrous elements of this layer are commonly arranged perpendicularly or obliquely at an acute angle to the arterial lumen, while the endothelial cells have long cytoplasmatic processes extending from the lower surface of the cell into the depth of the intima. At the same time, two animals were found to have

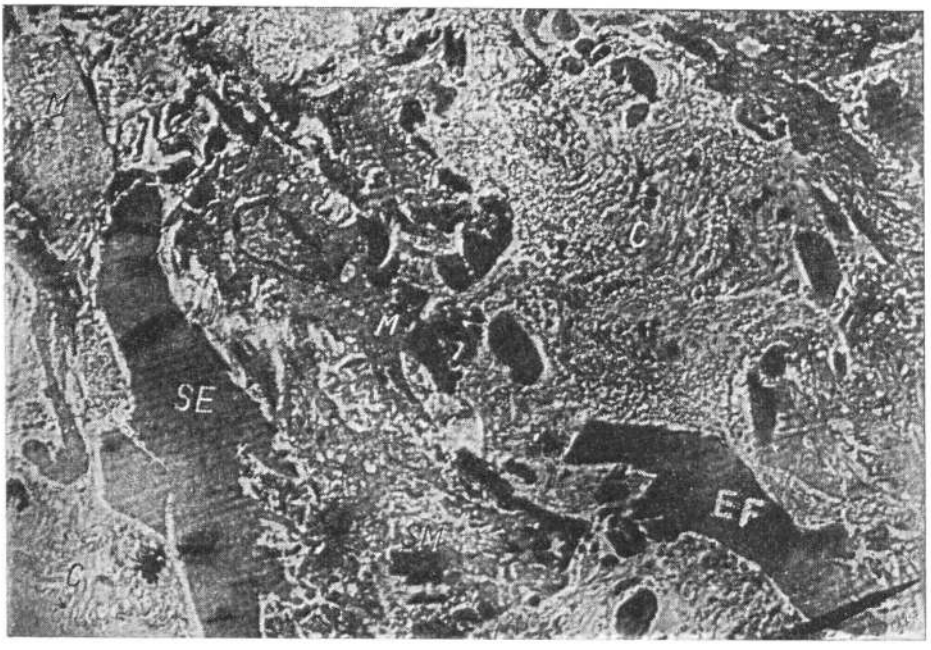

Fig. 5.

Swelling and irregular osmiophily of elastic fibres (*SE*) in the media of a monkey given cholesterol, thrombin, and pituitrin; *EF*—elastic fibres; *C*—collagen fibrils; *SM*—smooth muscle cells. Electron microphotograph. Magnification 4,000 times

a considerably increased number of elastic and collagenous fibres in the subendothelial layer of the aorta, which was accompanied only by a slight vacuolization of the endothelial cells.

In the media, under the cushion-like thickening of the intima and outside of it, one could also note split elastic fibres (Fig. 5). Also visible were elongated smooth muscle cells with a variable degree of vacuolization of the cytoplasm and the nucleus. In other preparations, collagenous fibres with an insignificant number of smooth muscle cells prevailed in certain portions of the media.

Changes in the elementary structure of the adventitia in experimental animals were roughly similar to changes in the identical fibres and cells noted in the media of the vessel.

These findings are interesting both from the viewpoint of inducing in monkeys vascular changes close to human atherosclerosis and for the study of the role of the accessory factors in its development. The morphological changes occurring in the initial stage of atherosclerosis are of great interest.

First of all, it should be noted that atherosclerotic changes were induced in the presence of an insignificant increase in the cholesterol content of the blood. This brings the conditions of the experiment closer to clinical conditions in which, as is known,

increased indices of the blood cholesterol level in developing atherosclerosis are encountered in 60-70 per cent of all cases (A. L. Myasnikov). It proved possible to provoke changes in the aorta in monkeys after the type of aortic atherosclerosis in rabbits and man against the background of unmarked changes in lipid metabolism but in the presence of changes in the blood coagulative system. As is known, a number of components taking part in blood coagulation or its prevention are closely associated with substances playing a major role in fat metabolism (for example, relationships of heparin to ß-lipoproteins). The role of the fibrin film and fibrin deposits in the mechanisms of atherosclerosis progression is also known (Friedman a. Byers). We believe that in addition to disturbances in lipid metabolism during induced atherosclerosis in monkeys an important role is played by changes in the coagulative properties of the blood and by an increase in the tone of the smooth muscles of the arteries, which intensifies and accelerates the progression of atherosclerosis. They are little known and attract the interest of experimenters and medical practitioners (E. I. Chazov).

The changes discovered by histological investigation are similar in quality to the above-described pathological symptoms seen in the early stages of experimental cholesterol atherosclerosis in rabbits.

The most likely mechanism of the development of such changes is, in our opinion, an increase in the permeability of the vessel wall attended with focal plasmorrhage as a result of dystrophic and necrobiotic processes in the elastic tissue of the aorta. It is apparent that under the conditions of an actual experiment an additional administration of pituitrin and thrombin only intensified the effect on the vessel wall, which was produced by administration of cholesterol alone. It should be also noted that the material under investigation made it possible to learn that focal dystrophic changes in the vessel wall precede and condition the subsequent development of lipoidosis, since the formation of "acute cushion-like plaques" takes place without an indispensable deposition of lipids in these portions of the intima.

Electron microscopy enables detection of a number of early structural changes in the elements of the aorta, which show the latter's active participation in the atherosclerotic process and are evidence against a passive lipid permeation of the aortic walls.

During experimental atherosclerosis dystrophic changes in the elastic fibres of the animal aorta or disturbances in the permeability of the vessel wall may occur in the first place. These manifestations are accompanied by destructive changes and oedema of the intima, and a change in the endothelial lining.

It is possible that the osmiophilic depositions on the endothelium evidence a change in the properties of its cellular surface. Such portions of the artery are apparently the site of subsequent cushion-like thickenings of the intima, which are microfocal plasmorrhages discoverable by light microscopy. Later, such thickenings become the site of accumulation of lipids which one fails to discover in the earlier initial changes in the aortic wall structure.

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THE MECHANISM OF ORIGINATION OF CORONARY MURMUR

I. I. SAVCHENKOV

In view of A. L. Myasnikov's conception of the preclinical period of vascular atherosclerosis there is need for elaboration of early diagnostics of the coronary disease in this period of its development.

As is known, atherosclerosis, including coronary atherosclerosis, often develops at a thriving age and may become clinically manifested after a prolonged latent period (5-10 years) as angina pectoris, myocardial infarction, atherosclerotic cardiosclerosis.

It is in place to note that in the years before distinct signs of coronary disease appear the patient's condition is often mistaken for mitral insufficiency on account of a systolic murmur. In this connection, it is pertinent to refer to the American cardiologist Milton Plotz who writes in his monograph *Coronary Disease* that myocardial infarction often develops in persons who already have murmurs, in particular a loud systolic murmur caused by sclerotic changes in the aorta, endocardium or fibrous ring.

We carried out special studies on the problem of sound phenomena in the heart. In x-ray-phonodiagnostic examination of coronary patients a systolic murmur often cannot be heard and recorded. But in some patients this murmur is detectable. During its special analysis by means of oesophageal endoauscultation we found this murmur to be audible not at the level of the left atrium but at that of the left ventricle, i.e., it was not due to regurgitation.

It is remarkable that after taking three drops of nitroglycerin this systolic murmur ceases and then recurs in 10-15 minutes' time. No x-ray and morphological changes in the heart and aorta are noted at the same time. All this indicates that in the given category of patients mitral insufficiency is absent and that its diagnosis is made erroneously as a result of incorrect interpretation of the systolic murmur. The latter occurring during anginose pains in the heart region should be apparently regarded as a manifestation of coronary disease.

For an accurate determination of the site and mechanism of systolic murmur during coronary disease we have carried out acoustic investigations of the heart during physiological experiments on dogs and hydraulic experiments on a cadaveric heart.

The experiments with the creation of a model with regulated extracorporeal coronary circulation carried out by A. V. Trubetskoi, a research worker of the Institute of Therapy, at M. E. Udelny's laboratory, proved helpful to the solution of our problem. During perfusion of a humorally isolated coronary system of a fibrillating heart we succeeded for the first time in detecting a murmur occurring in the phase of delivery of heparinized blood to the coronary vessels.

A phonogram of an exposed fibrillating heart shows oscillations of coronary murmur with a characteristic harmony of variations both in frequency and range (Fig. 1*A*). The same model of Trubetskoi was used for experiments with a normally pulsating heart. The difference, in the acoustic respect, consisted in the interference of sounds occurring during the delivery of blood to

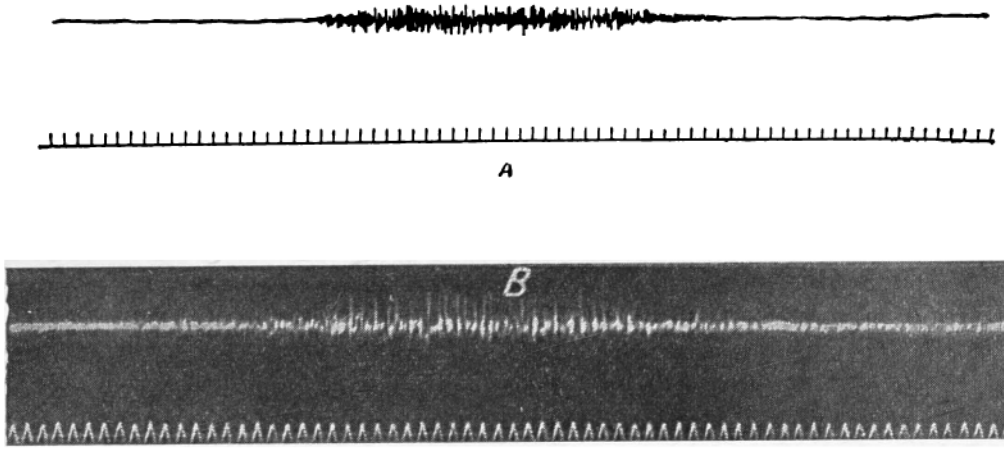


Fig. 1.

A—phonogram of coronary murmur. Upper curve—high-frequency oscillations of coronary murmur occurring during extracorporeal coronary circulation in an experiment; lower line—time mark (1/50).
B—interference of coronary murmur with sounds from a pulsating "empty" heart

the coronary vessels and the sounds caused by the systole of the pulsating "empty" heart. In such cases, in addition to the wide range of oscillations of tones, oscillations of a murmur of variable range and frequency were registered (Fig. 1*B*).

The murmur always appeared upon a rise in the arterial pressure from 100-200/50-60 mm Hg and higher. After an increase in the arterial pressure the murmur became longer and more intense. The frequency of coronary murmur was 100-150-200 cps. The range of oscillations was equal to 2-5 mm.

During an extracorporeal coronary circulation, the cardiac cavities were drained of blood. The venous blood supplied to the right atrium from the coronary veins was drained through a special cannula into the reservoir of the unit. This ruled out the conditions responsible for intraventricular murmur.

The cardiac sounds on the phonogram appeared only after defibrillation of the heart. The formation of tones in an empty pulsating heart may be attributed to oscillations of the valve apparatus due to the compressive force of the systole (*I* sound) and the absorptive capacity of the heart during diastole (*II* sound). Of course, the murmur (coronary) developing in the given experiment was not associated with the larger vessels, since the heart was completely isolated from the systemic and pulmonary circulation.

An exceptional role in the development of coronary murmur in the normal heart of the dog is played by the "superfluidity" of heparinized blood and a marked reduction in its viscidity. We have been able to prove on a hydraulic system of instruments the possibility of emergence of sound vibrations within the system of the coronary vessels of the cadaveric heart. Heparinized blood or physiological solution injected under pressure into the ostium of the left coronary artery cause sound vibrations in the coronary system. The intensity and frequency of these vibrations depend on the degree and speed of compression of the rubber bulb of the unit. The coronary murmur we have reproduced in the vessels of the cadaveric heart is the result not only of a faster flow of blood with decreased viscidity but also of a severe atherosclerotic change in the relief of the coronary walls and lumina (Fig. 2). In the coronary vessels affected with stenosis acoustic phenomena are manifested by high-frequency vibrations.

The anatomic and haemodynamic characteristics of the coronary blood supply system of the myocardium (relatively short distance from the ostium of the coronary arteries to the right atrial cavity; extensive capillary networks embracing each muscle fibre; pronounced pressure gradient between the aorta and the right atrium and the dynamic influence of the compressive force of the systole and the decompressive force of the diastole) enable the application of transmural electrokymography for a

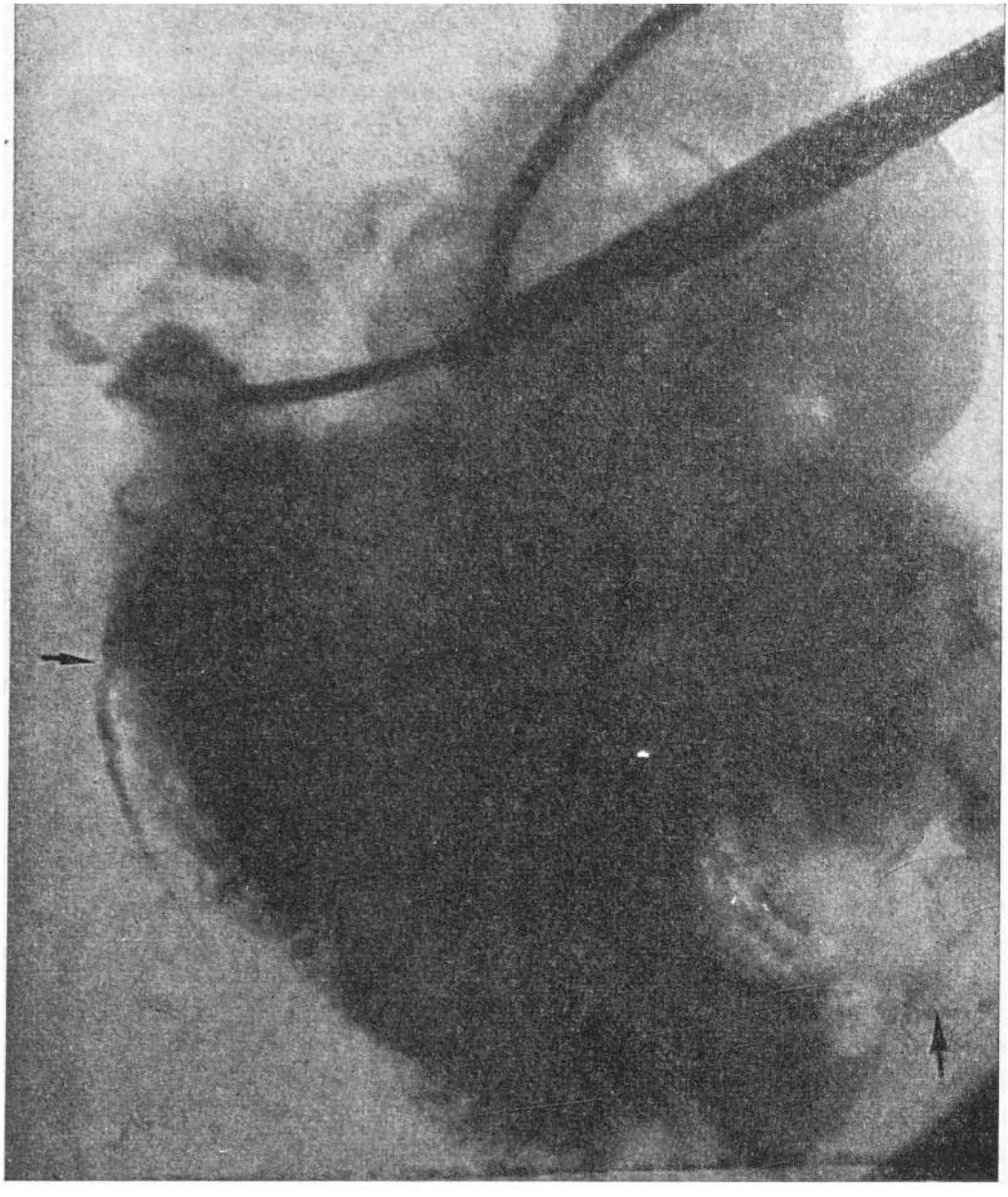
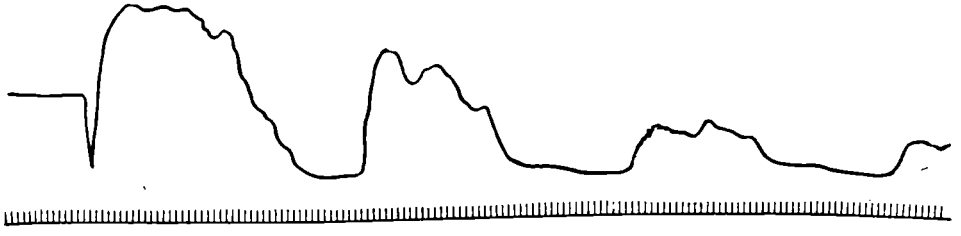


Fig. 2. Angiocardiogram of the coronary vessels in a cadaveric heart.

Stenosed parts of the coronary vessels (→) and collaterals in the cicatricial field of the myocardium are visible (↑)

Wall shadow

A



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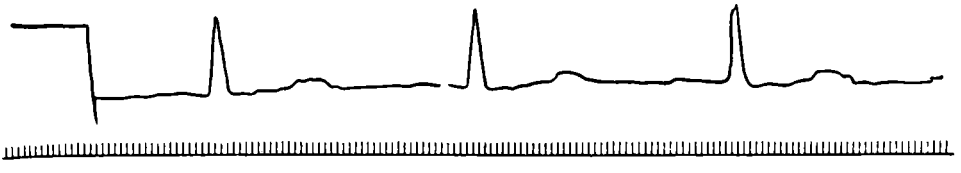

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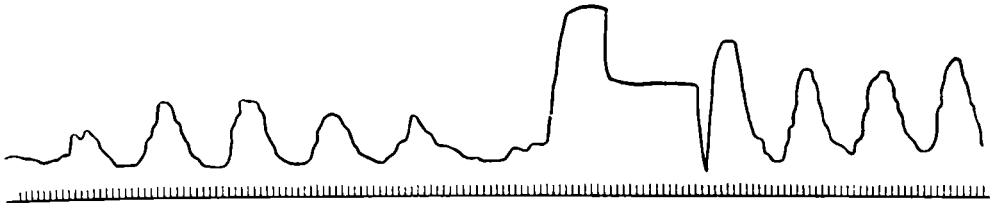

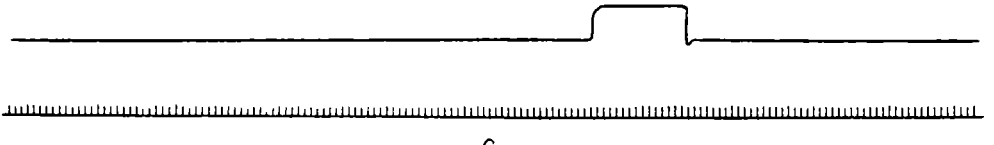

C

relatively precise determination of the blood current speed in the wall of the left ventricle.

The term "transmural electrokymography" implies a method for registering changes in the illumination of the fluorescent screen in the projection of the shadow of the left ventricular wall proper. The slit of the photocell should not overlap the external and internal contours of the ventricular wall, i.e., it should be located vertically, 5 mm inwardly to the pulsating contour of the left ventricle. This position of the photocell eliminates distortion occurring as a result of the rotary movement of the heart. We believe that transmural electrokymography reflects "influx" and "drainage" of blood in the coronary vessels. When interpreting a transmural electrokymogram one should bear in mind the specific features of the blood flow through the intramural coronary vessels: the compressive force of the systole contributes to the drainage, back flow of blood from the intramural vessels into the cavity of the right atrium, while the actively functioning diastolic system of the myocardium facilitates the influx of blood to the intramural vessels from the coronary vessels located on the heart surface (Fig. 3*A*). In stenosing coronarosclerosis and atherosclerotic cardiosclerosis such haemodynamic correlations are paradoxically changed, partly or totally (Fig. 3*B*). This is the advantage of transmural electrokymography. The latter was used to show plainly the influx and drainage of blood in the coronary arteries in experiments on a cadaveric human heart (Fig. 3*C*).

The geometric characteristics of transmural electrokymography of the heart (sharp projectional reduction in the range of pulsatory movements of the heart) rule out distortion of the contour displacement of the heart and pulsating change in the illumination of the shadowed mass of the heart in its cavitary projection.

The diagnostic value of x-ray electrokymophonography is the objective character of its data: the photocell sensing the variations in illumination of the shadow of the left ventricular wall graphically shows the amount of circulating blood, while the speed of the blood flow may be relatively determined by means

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Fig. 3.

A—transmural electrokymography; upper curve—transmural electrokymogram; lower curve—ECG; *B*—transmural electrokymography; upper curve—transmural electrokymogram in extracorporeal coronary circulation; lower curve—ECG; *C*—electrokymogram of cadaveric heart during forced delivery of sergosine through coronary vessels by means of hydraulic pump

of auscultation and phonography from the frequency and range of oscillations.

Changing the speed of the coronary blood flow, the amount of circulating blood and the amplitude of cardiac pulsations by one method or another, one can get an idea of the degree of predominance of the functional factor over the morphological factor of the coronary disease. Pharmacological tests must be very helpful in this respect. For example, if a slight systolic murmur of a uniform character ceases after a peroral administration of three drops of nitroglycerin, and in the transmural electrokymogram the range of the curve and configuration of the descending line return to normal this will point to predominance of the functional factor (spasm) over the morphological factor (sclerosis) in the coronary system. If, however, after the administration of three drops of nitroglycerin the intense high-frequency systolic murmur is reduced insignificantly and in the transmural electrokymogram the sloping curve of elevation is unchanged in amplitude this will be evidence of a marked predominance of the morphological factor over the functional one in coronary pathology.

Great importance in the differential diagnosis of coronary disease is increasingly attached to functional load tests.

In a patient with an impaired myocardial function, physical exertion (squatting ten times) is followed by a reduction in the intensity and length of coronary murmur; in patients with a relatively adequate myocardial function, however, this systolic murmur is noticeably increased.

Coronary murmur occurring in the clinical stage of coronary disease should be differentiated from that heard during stenosis of the aortic ostium, the ostium of the pulmonary artery and other defects, as well as during a strong influence of nervous and endocrine factors. For differentiation between coronary murmur in coronary disease and murmurs of another origin A. L. Myasnikov suggested analyses to be made of the frequency, range and time of occurrence of a murmur in account with the size of the heart, configuration and character of the pulsatory movement of the heart and larger vessels.

In analysis of the heart phonogram in a patient with diseased coronary vessels the eye is struck by a number of oscillations of identical form (decrescendo) occurring in the wake of the changed sound *I* and ending in the middle of the systole. The summing up of oscillations in murmurs of variable origin makes acoustic analysis extremely complicated.

Highly important for differentiation between the coronary murmur and the valvular murmur is an accurate x-ray determination of the size of the heart divisions and the character of car-

diac pulsations. As is known, in affections of the valve apparatus the cardiac sounds are substantially changed, which is uncharacteristic of the "coronary" murmur.

Special attention, as has been said above, should be given to the character of painful sensations in the heart region before and after nitroglycerin administration.

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DETERMINATION OF 17-OXYCORTICOSTEROIDS IN THE BLOOD PLASMA AND URINE IN CORONARY ATHEROSCLEROSIS

N. M. YUSHKOVA

Among the investigations on the problem of coronary atherosclerosis those concerning the function of the adrenal cortex have been attracting much interest during the past few years.

A number of authors (Donzelot, Kaufmann, Forsman et al., M. Z. Maisuradze) discovered an increase in the level of 17-ketosteroids and 11-oxysteroids of the urine in myocardial infarction and angina pectoris.

Meanwhile, other studies revealed a reduction in the level of these hormones of the adrenal cortex (I. G. Danilyak a. A.S. Loginov, L. A. Lushnikova).

During the study of 17-oxycorticosteroids in myocardial infarction and angina pectoris some authors discovered an increase in their level (Engel et al., Oka, E. A. Toloknova, I. Y. Usvatova), others a reduction (Maggi et al.).

The few available studies on adrenocortical hormones—17-ketosteroids, 11-oxysteroids in coronary atherosclerosis in the period between attacks of angina pectoris also present contradictory evidence: some authors point to an increase in hormonal secretion (E. A. Toloknova), others to a reduction of it (L. A. Lushnikova, M. Z. Maisuradze). The discrepancy between these findings is primarily due to the fact that examination of the adrenocortical function was carried out by different methods, in some cases only in the blood, in others only in the urine and, what is the most important, the majority of investigations were carried out without regard to the stages in the progression of coronary atherosclerosis and the pain syndrome—attacks of angina pectoris.

The purpose of the present research was to study the adrenocortical function in patients with different stages of coronary atherosclerosis by means of a simultaneous determination of 17-oxycorticosteroids in the blood plasma and the diurnal urine. The patients were examined both in the period of frequent attacks of angina pectoris and in the painless period.

A total of 70 patients with coronary atherosclerosis, 48 males and 22 females aged from 35 to 73 years were examined. In this group 21 patients had the disease in the 1st stage (according to A.L. Myasnikov's classification), 20 in the 2nd stage, and 29 in the 3rd stage. The control group consisted of practically healthy persons, 10 men and 10 women, aged from 37 to 66 years, without clinical signs of atherosclerosis.

17-oxycorticosteroids of which a considerable part consisted of hydrocortisone were determined by Porter and Silber's method in N.A. Yudayev's and Y.A. Pankov's modifications for the blood plasma and in M. A. Krekhova's modification for the urine.

It was found that the level of 17-oxycorticosteroids of the blood in practically healthy men was 11.56±0.56 γ per 100 ml of plasma on the average (see table). The level of free 17-oxycorticosteroids in the diurnal amount of urine was equal to 0.207±±0.2 mg, that of the total ones being 3.59±0.17 mg. In women, the level of 17-oxycorticosteroids was 8.72±0.63, 0.163±0.01 and 2.61±0.08 respectively. Thus, free 17-oxycorticosteroids of the urine constituted 5.7 per cent and 6.2 per cent of the total ones in men and women respectively.

Examination of patients in the 1st and 3rd stages of coronary atherosclerosis in the absence of attacks of angina pectoris showed that the level of 17-oxycorticosteroids in the blood plasma and the diurnal amount of urine was within physiologically normal limits in most cases; only in several individuals was an insignificant decrease noted (Figs. 1, 2).

For example, in men with coronary atherosclerosis in the 1st stage the average level of 17-oxycorticosteroids in the painless period was 11.03±0.55 γ per 100 ml of plasma, in the 3rd stage 10.19±0.54 γ. The level of free 17-oxycorticosteroids of the urine in the 1st stage was equal on the average to 0.205±0.02 mg, of the total ones to 3.46±0.21 mg; in the 3rd stage 0.164±0.01 mg of free and 3.52±0.18 mg of total ones. In women in the 1st stage the average level of 17-oxycorticosteroids in the painless period was 9.27±0.82 γ per 100 ml of plasma, in the 3rd stage—8.85±0.96 γ. The level of free 17-oxycorticosteroids of the urine in the 1st stage was equal to 0.120±0.015 mg, of the total ones to 2.39±0.2 mg; in the 3rd stage—0.150±0.03 and 2.57±0.09 mg respectively (see table).

Statistical processing of these findings for the sake of comparison with the results of examination of healthy persons did not reveal any significant difference.

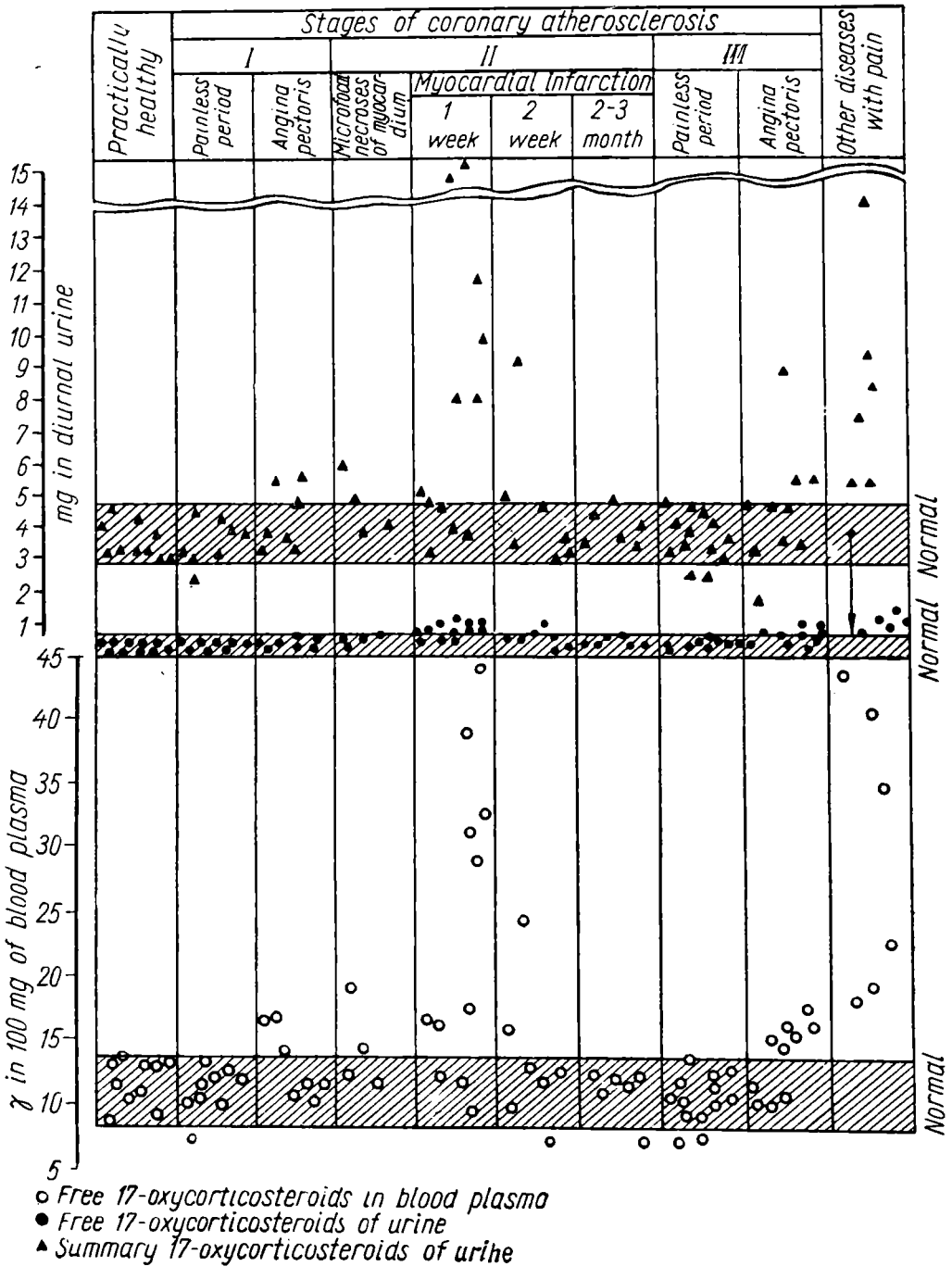

Fig. 1. The 17-oxycorticosteroid content of the blood plasma and the diurnal urine in men with coronary atherosclerosis and in control groups.

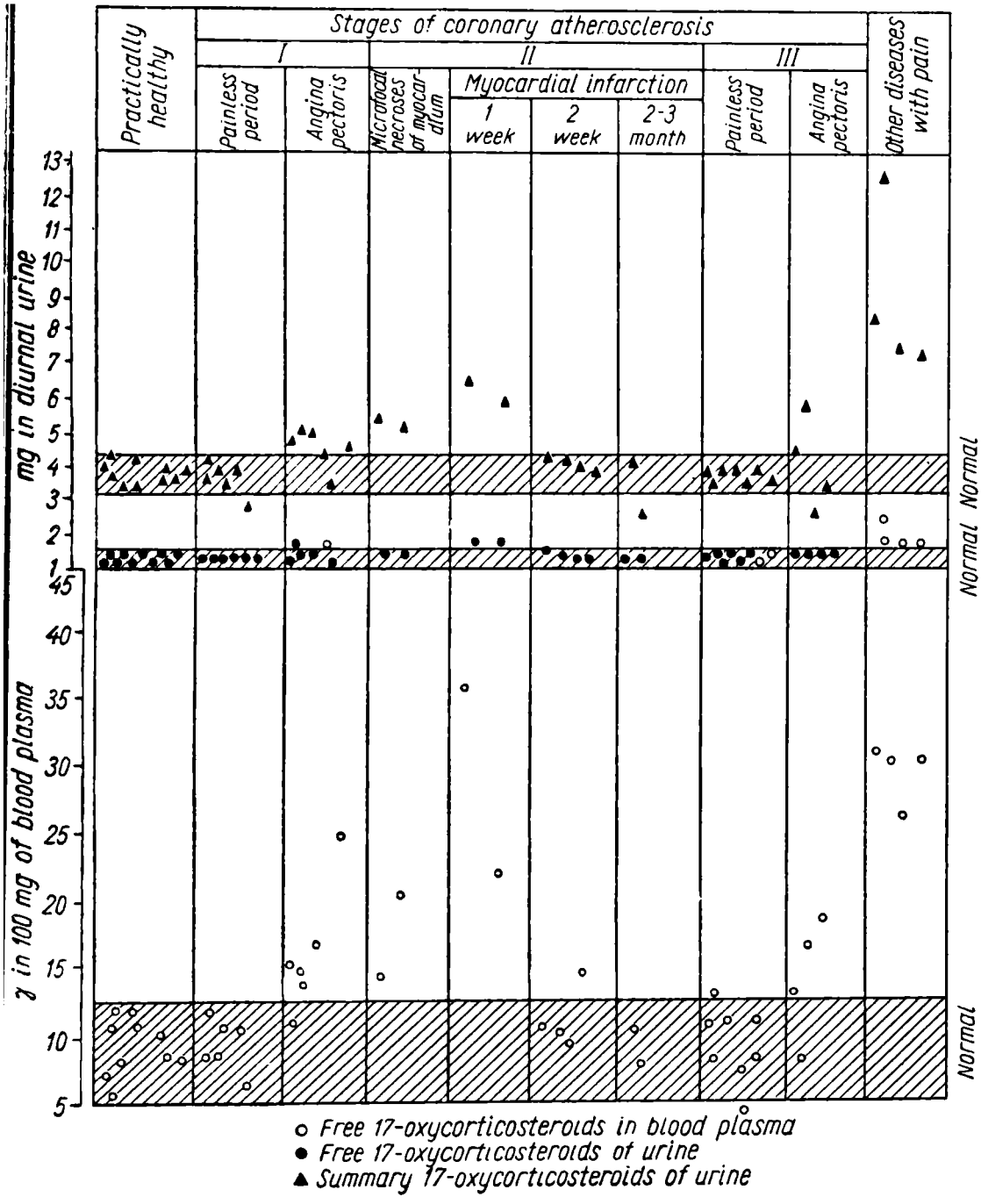

Fig. 2. The 17-oxycorticosteroid content of the blood plasma and the diurnal urine in women with coronary atherosclerosis and in control groups.

AVERAGE INDICES OF 17-OXYCORTICOSTEROID CONTENT OF PLASMA AND URINE IN CORONARY ATHEROSCLEROSIS (70 PATIENTS) AND IN CONTROL GROUPS (20 HEALTHY PERSONS) AND IN 10 PERSONS WITH VARIOUS DISEASES ATTENDED WITH ATTACKS OF PAIN

	Sex	Practically healthy	Stages of Coronary Atherosclerosis								Other diseases with pain
			I			II			III		
			Painless period	Angina pectoris	Microfocal myocardial necroses	Myocardial infarction			Painless period	Angina pectoris	
						1st week	2nd week	2nd-3rd month			
17-oxycorticosteroids in blood plasma, γ	m	11.56	11.03	13.12	14.25	22.78	13.51	10.87	10.19	13.90	29.40
	f	8.72	9.27	15.72	17.00	28.60	10.75	8.70	8.85	13.70	29.10
Free 17-oxycorticosteroids of urine, mg	m	0.207	0.205	0.190	0.391	0.610	0.382	0.170	0.164	0.310	1.176
	f	0.163	0.120	0.214	0.264	0.580	0.143	0.135	0.150	0.260	0.600
Total 17-oxycorticosteroids of urine, mg	m	3.59	3.46	4.23	4.51	8.04	4.36	3.78	3.52	4.16	8.05
	f	2.61	2.39	3.32	4.14	5.07	2.82	2.20	2.57	2.82	7.90

Examination of patients in all stages of coronary atherosclerosis during attacks of pain in angina pectoris revealed an increase in the secretion of 17-oxycorticosteroids in the majority of cases. It was noted at the same time that the increase in the 17-oxycorticosteroid content of the blood plasma or the urine in myocardial infarction was greater than in angina pectoris (Figs. 1, 2).

For example, in men with angina pectoris in the 1st stage of coronary atherosclerosis the average 17-oxycorticosteroid content of the blood plasma was equal to 13.12 ± 0.96 γ per 100 ml of plasma, in the 3rd stage—13.90 ± 0.94 γ per 100 ml of plasma. The level of free 17-oxycorticosteroids in the urine in the 1st stage was 0.190 ± 0.03 mg, of the total ones—4.23 ± 0.35 mg; in the 3rd stage—0.310 ± 0.04 and 4.16 ± 0.66 mg respectively.

In women in the 1st stage of coronary atherosclerosis the average 17-oxycorticosteroid content of the blood plasma during angina pectoris was 15.72 ± 1.63 γ per 100 ml of plasma, in the 3rd stage—13.70 ± 2.32 γ per 100 ml of plasma. In the 1st stage the level of free 17-oxycorticosteroids of the urine during angina pectoris was equal to 0.214 ± 0.07 mg, of the total ones to $3.32 \pm \pm 0.25$ mg; in the 3rd stage—to 0.260 ± 0.027 and 2.82 ± 0.75 mg respectively.

In the first week of myocardial infarction the 17-oxycorticosteroid content of the blood plasma was on the average 22.78 ± 2.97 in men and 28.60 ± 6.59 γ per 100 ml of plasma in women. The level of free 17-oxycorticosteroids in the urine of men was equal to 0.610 ± 0.07 mg, of the total ones to 8.04 ± 1.5 mg; in women to 0.580 ± 0.05 and 5.07 ± 0.33 mg respectively.

In cases with microfocal necroses of the myocardium we also discovered an increase in the level of 17-oxycorticosteroids, which, however, was less significant than that seen in macrofocal myocardial infarction, as described above.

Further observations on patients with myocardial infarction and investigations performed in the 2nd week showed that in the absence of pains the 17-oxycorticosteroid content of the blood plasma and diurnal urine in most cases returned to normal. The normal level of 17-oxycorticosteroids was in the majority of patients found $2\text{-}2^1/_2$ months after myocardial infarction.

For analysis of the character of increase in the level of adrenocortical hormones a study was undertaken on a group of 6 men and 4 women aged from 23 to 75 years having various diseases attended with attacks of pain (gastric ulcer, renal colic, hepatic colic, etc.). They, just as the patients with coronary atherosclerosis, were examined in the period of pain attacks for the 17-oxycorticosteroid content of the blood plasma and diurnal urine.

It was found that this period was marked by a considerable

increase in the level of hormones, this increase being noted during a severe pain syndrome not only in young but also in old persons, for example, in a 75-year-old woman with renal colic. Thus, the average 17-oxycorticosteroid content of the blood plasma was equal to 29.40±4.55 γ in men and to 29.10±1.24 γ per 100 ml of plasma in women. The level of free 17-oxycorticosteroids in the diurnal amount of urine in men was on the average 1.17±±0.49 mg, of the total ones 8.05±1.26 mg; the respective figures for women were 0.600±0.16 and 7.9±1.48 mg.

Thus, the average increase in the level of 17-oxycorticosteroids in persons with various diseases during attacks of pain is similar to that seen in myocardial infarction.

Consequently, it is not the character of the disease but the presence of pain that determines the participation of the adrenal cortex in the general reaction of the body.

CONCLUSIONS

1. The 17-oxycorticosteroid content of the blood plasma and diurnal urine of patients with coronary atherosclerosis in the 1st and 3rd stages in the absence of attacks of angina pectoris does not exceed the normal physiological limits.

2. The level of hydrocortisone during frequent attacks of angina pectoris in the 1st and 3rd stages of coronary atherosclerosis, as well as in the acute period of myocardial infarction in the 2nd stage of coronary atherosclerosis exceeds normal limits in the majority of cases, this increase being larger in myocardial infarction than in angina pectoris. By the 8th day of the disease, the level of adrenocortical hormones in the blood plasma and urine returns to normal in the majority of patients with myocardial infarction.

3. For analysis of the character of increase in the level of adrenocortical hormones a group of persons with various diseases (gastric ulcer, nephrolithiasis and cholelithiasis, etc.) were examined in the period of attacks of pain. In all cases, a distinct increase in the level of 17-oxycorticosteroids was found.

4. The increase in the level of adrenocortical hormones during coronary atherosclerosis is a non-specific reaction to pain and may be interpreted as evidence of the participation of various systems in the general reaction of the body to a strong stimulus.

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RELATIONSHIP OF CORONARY THROMBOSIS TO MYOCARDIAL INFARCTION

A. M. VIKHERT, V. N. GUDIM, V. B. BOGUSLAVSKY

The problem of the role played by coronary thrombosis in the development of myocardial infarction cannot be considered finally settled so far. As it is pointed out by A.L. Myasnikov, the problem of myocardial infarction cannot be reduced to coronary thrombosis and the latter is not a decisive factor in the pathogenesis of this disease.

Various authors sometimes hold diametrically opposite views of this problem, while the data found in the literature on the relationship between coronary thrombosis and myocardial infarction and on the rate of coronary thrombosis in cases of sudden death in myocardial infarction are contradictory.

Some authors believe that coronary thrombosis in the majority of cases concurs with or shortly follows myocardial infarction. Others adhere to the opposite view regarding thrombosis as the principal factor responsible for myocardial infarction.

I.V. Davydovsky points out that although coronary thrombosis is encountered in 70-80 per cent of cases of myocardial infarction, the latter is by no means necessarily linked with arterial thrombosis and that this thrombosis is often a concurrent or subsequent phenomenon. A.V. Smolyannikov and A.K. Apatenko carried out a statistical analysis of 305 cases of myocardial infarction and histologically studied the hearts of 38 persons who had died of coronary insufficiency and myocardial infarction. They found thrombosis of the larger branches of the coronary arteries only in 31.2 per cent of cases. These authors believe that coronary thrombosis occurs simultaneously with or

subsequent to myocardial infarction and simply lends permanence to the existing disturbance in the coronary circulation.

A.V. Arkhangelsky points out that although coronary thrombosis is encountered in two out of every three cases of myocardial infarction, the principal role in its origination is played by functional disturbances; he admits the possibility of thrombus formation when myocardial infarction has already occurred.

On the contrary, N.N. Anichkov and his research group maintain that coronary thrombosis is the principal pathogenetic factor responsible for myocardial infarction. According to their evidence, coronary thrombosis was found in 75 per cent of the fatal cases of myocardial infarction, although in their personal 36 observations they discovered thrombosis only in 23, i.e., in approximately 65 per cent of the cases.

Similar evidence has been furnished by L.G. Shamesova; in her observations, coronary thrombosis was found in 83 out of 103 fatal cases due to an acute disturbance in the coronary circulation (i.e., in 80.5 per cent) and in 17 out of 30 fatal cases at a later time (56.7 per cent). From a comparison drawn between the thrombus site and the topography of myocardial infarction the author inferred that coronary thrombosis occurs prior to myocardial infarction and is the latter's cause.

E.M. Helstein also believes that thrombosis is a primary condition with regard to myocardial infarction. In his opinion, the origination of myocardial infarction depends not only on the size of the thrombus but also on the speed of its formation.

S.G. Moiseyev reports the presence of coronary thrombosis in 41.7 per cent of cases of myocardial infarction; A.I. Strukov and his research group discovered thrombosis in 70.4 per cent of cases of hypertension and in 72.7 per cent of atherosclerosis. According to P.E. Lukomsky's evidence, thrombosis was detected in 105 out of 179 fatal cases of myocardial infarction, i.e., in 58.6 per cent of cases. The American Committee for Anticoagulants reported coronary thrombosis in 74.2 per cent of cases of myocardial infarction.

Thomas and Lee consider that all myocardial infarctions are associated with thrombosis of the system of the coronary arteries.

Papacharalampous and Zollinger report the presence of thrombosis in 54.4 per cent of 126 cases of acute infarction; according to Saphiro, thrombosis was found in 71.1 per cent of cases (in 32 cadavers out of 45).

In several other studies the authors considered the incidence of thrombosis in relation to the patient's survival time. According to N.G. Shulyakovskaya, patients who died within two hours of the onset of angina pectoris had no thrombosis but half of those surviving longer than 24 hours were afflicted with it.

A.V. Smolyannikov also points to a growth in the rate of thrombosis in connection with an increase in life span.

Spain and Bradess have inferred that thrombosis is a secondary condition on account of the fact that among persons who died within the first hour of the onset of angina pectoris the rate of thrombosis was $3^1/_2$ times as low as that among those who had died after 24 hours (16 per cent and 54 per cent respectively).

The objects of study in the present research were as follows: (1) associations between sudden death, myocardial infarction and coronary thrombosis; (2) significance of thrombosis in the clinical course of myocardial infarction; (3) influence of anticoagulant therapy on the rate of coronary thrombosis in myocardial infarction.

The study was made on fatal cases of acute heart failure reported by the Lefortovo mortuary of the Moscow city medico-legal board for the period from 1951 through 1961 (1,026 cases) and fatal cases of myocardial infarction within the first twenty days of the appearance of clinical symptoms according to reports of the dissecting rooms at the Institute of Therapy under the USSR Academy of Medical Sciences, the 18th, 30th and 42nd city hospitals for the period 1960-1962, the 1st, 2nd and 23rd city clinical hospitals and the Sklifosovsky Institute for the period from 1950 through 1961 (788 cases). Listed as sudden death were fatal cases of apparently healthy persons within a period of several minutes to six hours after a heart attack, i.e., in the period when the morphological signs of myocardial infarction are still unmarked at autopsy. A histological study was made on all cases of myocardial infarction handled by the dissecting rooms of the Institute of Therapy under the USSR Academy of Medical Sciences and the 18th, 30th and 42nd city hospitals (104 observations).

The distribution of cases according to nosological forms, sex, age and degree of coronary stenosis is presented in Table 1.

As is seen in the table, infarction occurred in men almost twice as often as in women (62.3 per cent and 37.7 per cent respectively); coronary thrombosis was also more frequent among men (44 per cent) than among women (34.3 per cent). There is a considerable difference in regard to age: in women under 40 infarction is not found at all, at the age from 40 to 49 years it is infrequent (2 per cent of all cases), in the seventh decade of life the infarction rate is highest (82.9 per cent), whereas men under 40 account for 2.4 per cent of all cases of infarction, 10.2 per cent of cases being observed in the fifth decade of life, 31.2 per cent in the sixth, and 56.1 per cent in the seventh, i.e., in men myocardial infarction occurs at a younger age (by ten years).

Table 1

GENERAL CHARACTERISTICS OF MYOCARDIAL INFARCTION ACCORDING TO NOSOLOGICAL FORMS, SEX, AGE, RATE OF THROMBOSIS, AND DEGREE OF CORONARY STENOSIS*

	Men			Women		
	491 cases of infarction, 216 with thrombosis (44%)			297 cases of infarction, 102 with thrombosis (34.3%)		
	cases of infarction	with thrombosis	without thrombosis	cases of infarction	with thrombosis	without thrombosis
Hypertension	219 (44.6%)	200	119	116 (39.1%)	50	66
Atherosclerosis	272 (55.4%)	116	156	181 (60.9%)	52	129
Age						
20-29 years	1 (0.2%)	—	1	—	—	—
30-39 years	11 (2.2%)	5	6	—	—	—
40-49 years	50 (10.2%)	25	25	6 (2%)	4	2
50-59 years	154 (31.2%)	72	82	45 (15.1%)	20	25
60 years and older	275 (56.1%)	114	161	246 (82.9%)	78	168
Coronary stenosis by over 50% of lumen dia.	347 (70.5%)	147	200	256 (86.2%)	81	175

* Total cases of infarction—788, 318 with thrombosis (40.3%)

In the overwhelming majority of cases infarction with and without thrombosis develops in the presence of marked atherosclerotic changes manifested by coronary stenosis, contraction of the vessel lumen by more than a half being noted among men in 70.5 per cent and among women in 86.2 per cent of cases. The somewhat higher rate of stenotic processes in women is possibly due to the prevalence of elderly persons in this group. It also follows from Table 1 that in the total number of cases of myocardial infarction the rate of thrombosis was equal to 40.3 per cent. This figure, however, does not show the actual state of affairs, since it illustrates observations without regard to the time factor, namely the term of survival of patients with myocardial infarction.

We consider it wiser to regard the distribution of thrombosis in myocardial infarction in relation to the life span of patients

and compare these data to the proportion of thromboses among cases of sudden death due to myocardial infarction. The above-mentioned data are presented in Table 2.

Table 2

DISTRIBUTION OF FATAL CASES OF MYOCARDIAL INFARCTION WITH AND WITHOUT THROMBOSIS IN RELATION TO THE TERM OF SURVIVAL

Distribution according to groups	Term of survival							
	Sudden death (within 6 hr)	Within 12 hr	Within 24 hr	Within 2 days	Within 3 days	Within 4 days	Within 5-6 days	Within 7 to 20 days
With thrombosis	43	43	20	30	32	34	38	121
Without thrombosis	983	168	63	52	29	25	32	101
Proportion of thrombosis, %	4.1	20.4	24.1	36.5	52.4	57.6	52.3	54.5

As is seen from the above table the rate of coronary thrombosis among fatal cases of acute coronary insufficiency within the first six hours is 4.1 per cent, but as early as within the first 6-12 hours the proportion of thromboses grows to 20.4 per cent and subsequently continues to increase, reaching 24.1 per cent in fatal cases in the period from 12 to 24 hours and 36.5 per cent in fatal cases within two days and 52.4 per cent if death occurred within three days. Subsequently, with an increase in the life span the proportion of coronary thromboses remains at approximately the same level, varying within narrow limits. These findings indicate that in the overwhelming majority of cases thrombosis is secondary with regard to acute ischaemia and myocardial infarction, since it is usually absent in the early stages of this disease.

Histological studies and comparisons of the term of infarction and coronary thrombosis in a number of observations also serve to confirm the above proposition: in certain cases, long-standing myocardial infarction with a marked leukocyte reaction or signs of organization in the coronary arteries is found to be concurrent with a fresh thrombus without any signs of organization, i.e., there is an obvious discrepancy between the time of the onset of infarction and thrombosis; analogous observations have also been reported by S.Kh. Khamitov.

The secondary character of thrombosis is confirmed also by certain clinical and morphological findings. For example, there have been a number of cases when the first infarction for which the patient had been hospitalized was in 5-15 days' time fol-

lowed by another attack of angina pectoris with a fatal outcome within a period of several hours to 2-3 days. In such cases autopsy revealed myocardial infarction with marked signs of organization coinciding in time with the first attack of angina pectoris without a thrombus in the coronary artery and a fresh red thrombus in one of the coronary arteries without signs of organization, with another fresh myocardial infarction or without it (if death occurred within several hours of a repeated attack of angina pectoris). One of such observations is given here for illustration.

Patient G., aged 61, was admitted to the Institute of Therapy on June 16, 1961, for myocardial infarction. During the next twenty days the patient had repeated severe attacks of angina pectoris; on June 14, electrocardiography revealed myocardial infarction (earlier no ECG had been taken). At the Institute the patient suffered from repeated attacks of pain in the heart region which were relieved by promedol injections; other symptoms were distended abdomen, hiccough and vomiting. On June 19, at 9.50 p. m. the patient died. Autopsy findings were as follows (autopsy record No.28): in the pericardial region 750 g of liquid blood and blood clots. The posterior wall of the left ventricle was bulging somewhat: its median part, closer to the left heart margin, showed a rupture about 1.5 cm in length, with rugged edges. In cross-section the tissue of the posterior wall of the left ventricle had a motley appearance; in the lower part down to the apex, involving the posterior portion of the septum, were noted prolapsed parts of a greyish-yellow colour, surrounded with a red border; higher up, in the posterior wall there was an extensive area of a reddish-yellow colour with alternating dark-red portions, at places with haemorrhages along the periphery. This region was of flaccid consistency and corresponded to the bulging part of the posterior wall. Also here, closer to the left edge of the heart was the above-mentioned perforating rupture of the ventricular wall. The lumina of the left descending and circumflex rami were free but narrowed owing to the presence of atherosclerotic plaques. In the middle third of the right circumflex ramus, there was a loose red thrombus seated on a small atherosclerotic plaque. Microscopic examination closer to the apex revealed a field of necrosis of muscular tissue with signs of organization (Fig. 1); higher up was a fresh infarction of 2-3 days' duration with a slight leukocyte reaction along the periphery (Fig. 2). In the lumen of the artery there was a fresh thrombus without signs of organization (Fig. 3).

Thus, the patient, during one of angina pectoris attacks which had occurred before his admission to the Institute, developed myocardial infarction involving the lower part of the posterior wall of the left ventricle. This infarction was diagnosed by means of electrocardiography on June 14; the first infarction had existed approximately for 10-14 days. During his stay at the Institute the patient developed thrombosis of the right coronary artery, accompanied by another, fresh extensive infarction of the posterior wall, with subsequent rupture of the wall and tamponade of the pericardium which was the immediate cause of death.

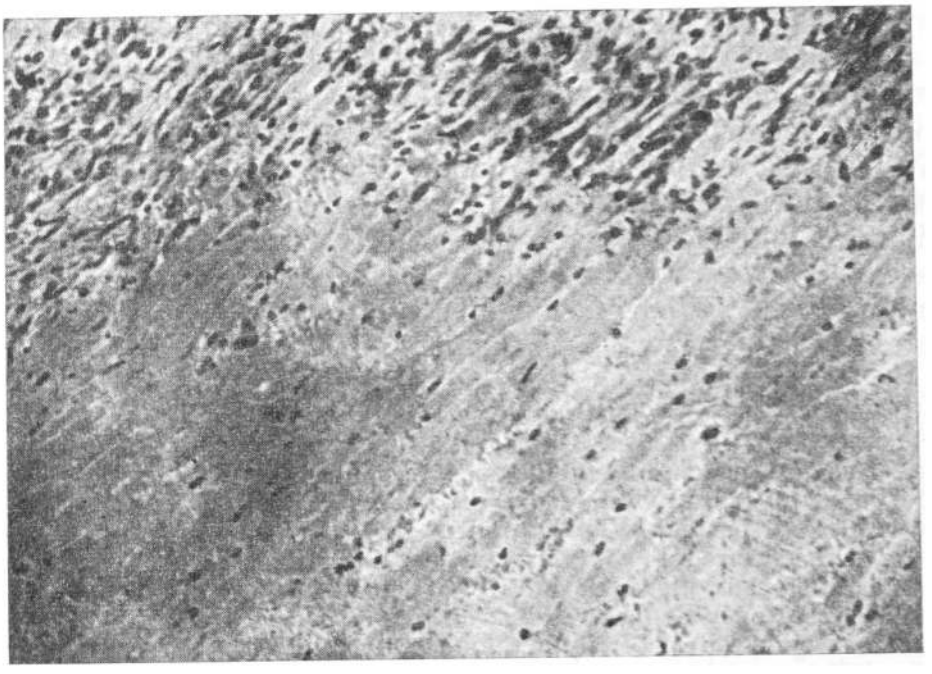

Fig. 1. Field of necrosis of muscle tissue with signs of organization. Stained with hematoxylin-eosin. Magnification 140 times

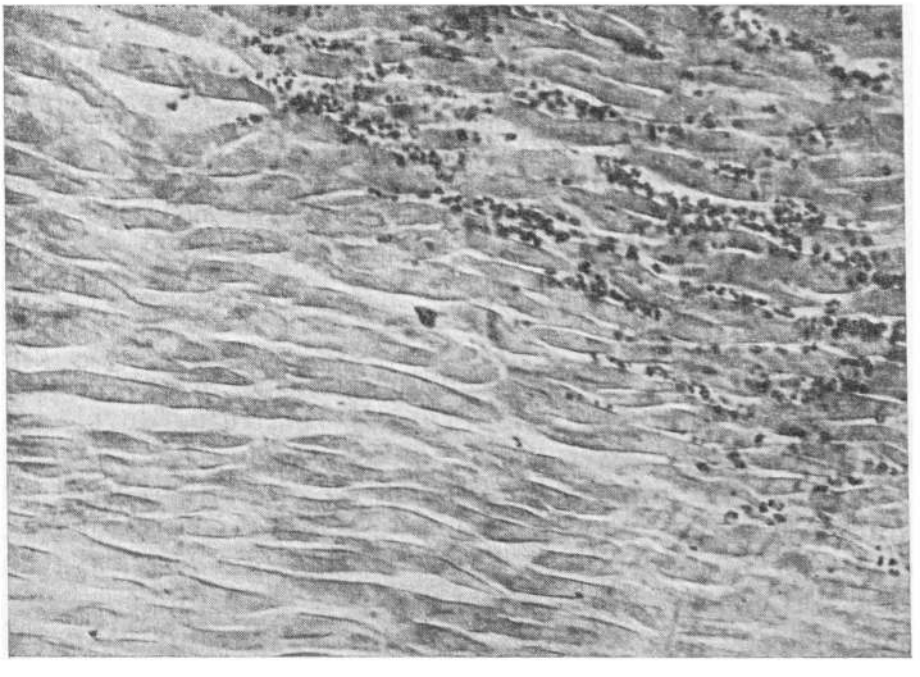

Fig. 2. Fresh infarction of 2-3 days' duration with a slight leukocytic reaction on the periphery. Stained with hematoxylin-eosin. Magnification 140 times

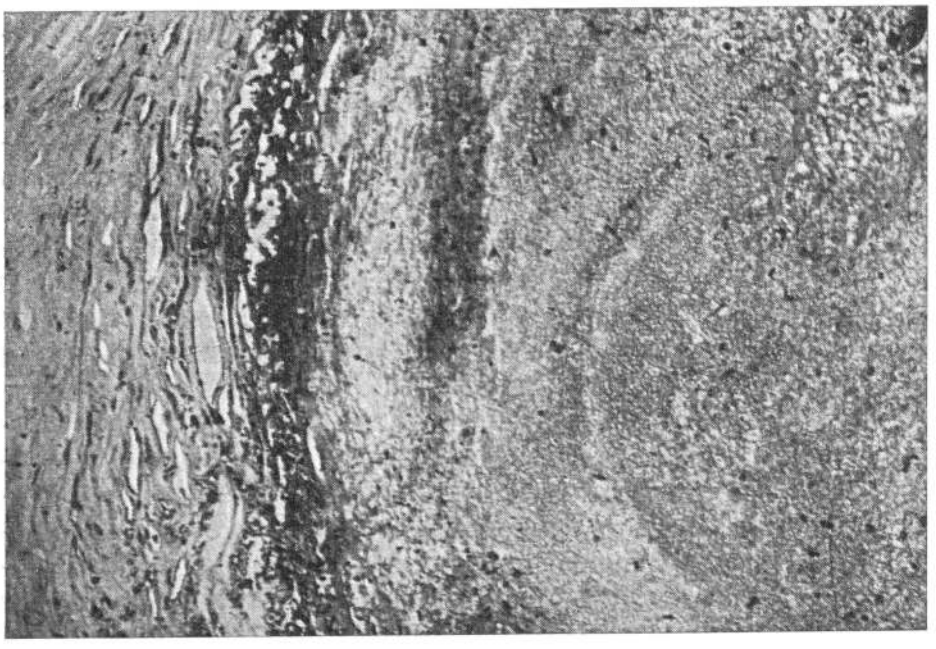

Fig. 3. A fresh thrombus in the arterial lumen with no signs of organization. Stained with hematoxylin-eosin. Magnification 140 times

We have at our disposal another 36 analogous reliable observations, which adds up to 11.3 per cent of the total rate of infarction with thrombosis. It can also be presumed that a considerable number of cases of the so-called myocardial infarction with a protracted course are due precisely to an accompanying secondary coronary thrombosis and the formation of new necrotic foci in the heart muscle. Apparently, the incidence of such cases is considerably higher than that we have reported, but during a retrospective analysis of case histories and records of pathoanatomical examination we met with a number of difficulties caused by the absence of a purposeful approach on the part of clinicians and pathoanatomists and by insufficiently accurate records.

In examination of a separate group of fatal cases of myocardial infarction at the Institute of Therapy under the USSR Academy of Medical Sciences one's attention is drawn to the low rate of thrombosis among them: among 39 patients who died at a later time (5th-20th day) thrombosis was found only in seven (17.9 per cent), i.e., in the period when the proportion of thrombosis was over 50 per cent. Thus, at the Institute of Therapy the incidence of thrombosis was three times less compared to the average rate. We attribute this difference entirely to the anticoagulative therapy applied extensively at the Institute of Therapy immediately upon admission of a patient. It evidently

prevented secondary thrombus formation in the coronary arteries. In this respect our findings agree with the clinical observations by E.I. Chazov of the Institute of Therapy, who discovered in a considerable part of patients with myocardial infarction, particularly those admitted with a pain syndrome, an increase in fibrinolytic activity in early hours with its subsequent sharp reduction (from the end of the first day of the disease), which was ultimately accompanied by an increase in the coagulability of the blood. Naturally, one wonders about the causes of secondary thrombus formation in the coronary arteries during myocardial infarction.

According to current views, the mechanism of blood coagulation is connected with a dynamic balance between the coagulative (thromboplastic) and anticoagulative (thrombolytic) systems. A disturbance in the coagulative process may be due either to an increase in thromboplastin or a decrease in thrombolytic activity. As is known, the formation of thrombin takes place under the influence of thromboplastin in the presence of calcium ions. The source of tissue thromboplastin, according to Astrup, may be any dead tissue, including necrotized tissue of the myocardium capable of a comparatively high thromboplastin activity. Hence it can be presumed that coronary thrombosis in myocardial infarction is to a certain extent due to the above-mentioned disturbances in the coagulative mechanisms, since the necrotized portion of the heart muscle becomes the source of thromboplastin, and this in its turn leads to an increase in blood coagulability. Possibly, the cause of secondary thrombosis is more intricate. It is difficult to clarify it because of the current inadequate understanding of the significance of changes in the coagulative system during thrombosis.

As has been noted above, E.I. Chazov showed the presence of a protective anticoagulative reaction within the early hours of the onset of infarction. This may interfere with thrombus formation and possibly contribute to lysis in its initial stages. The subsequent inhibition of the anticoagulative mechanisms, however (decrease of fibrinolytic activity), provides the conditions required for secondary thrombus formation. This is confirmed by the fact that in patients accommodated at the Institute of Therapy thrombi of the coronary arteries, if they formed at all, appeared at later periods, i.e., upon the completion of heparin and fibrinolysin therapy.

It is quite obvious that an important role is played by the condition of the body in which develop disturbances of the coagulative system, namely haemodynamic disorders, decrease of the blood pressure with deceleration and reduction of the blood current in coronary arteries with atherosclerotic lesions.

Briefly summing up, the cause, significance and place of thrombosis of myocardial infarction may be presented as follows.

Acute ischaemia and myocardial infarction are basically connected with functional disturbances in the coronary circulation occurring under the influence of physical load, neuro-humoral, emotional and other factors usually in the presence of a corresponding morphological foundation in the form of stenosing atherosclerosis of the coronary arteries. In the overwhelming majority of cases coronary thrombosis is not apparently the primary cause of ischaemia and myocardial infarction.

Coronary thrombosis is a secondary condition consequent upon a disturbance in the coagulative and anticoagulative systems. The accompanying secondary thrombus, however, stabilized the existing disorders in the coronary circulation on the one hand and leads to the formation of new foci of ischaemia on the other hand. These may be the immediate cause of death or make infarction run a protracted course.

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THE ORIGIN OF ELECTROCARDIOGRAPHIC SIGNS OF MYOCARDIAL INFARCTION AND THEIR DIAGNOSTIC VALUE IN THE LIGHT OF MODERN ELECTROPHYSIOLOGY OF THE HEART

M. G. UDELNOV

The most widespread theory explaining the pathogenesis of the ECG signs of myocardial infarction has hitherto been Wilson and Beyley's theory according to which every ECG sign taken separately is determined by its specific conditions successively developing in ischaemia. At the same time, Beyley and his followers (V.E. Nezlin, Rushmer) regard ischaemia as the only specific factor producing inversion and the "giant" *T* wave as well as displacement and the arched shape of the *S-T* segment. The pathological *Q* wave and *QS* wave are in effect attributed by them to the passive electrical properties of necrotized tissue and the scar, pointing out at the same time that an indispensable condition of registration of ECG changes is a deep penetration of the lesion focus (or aneurysm) through the thickness of the myocardial wall.

In the course of our investigations it has been found in recent time that necrotized tissue forming as a result of ischaemia takes an active part in the formation of ECG signs of myocardial infarction by influencing the bioelectrical and functional properties of intact myocardial structures (M.G. Udelnov).

Although the focus of necrosis forms as a result of ischaemia its action on the adjoining intact myocardium manifests itself irrespective of ischaemia.

The influence of the necrotized tissue proper on the myocardium was studied in our experiments using imposition of necrotized tissue on the surface of an intact myocardium with a normal blood circulation. It was found under these conditions that the effect produced by necrotized tissue paralyses the bioelec-

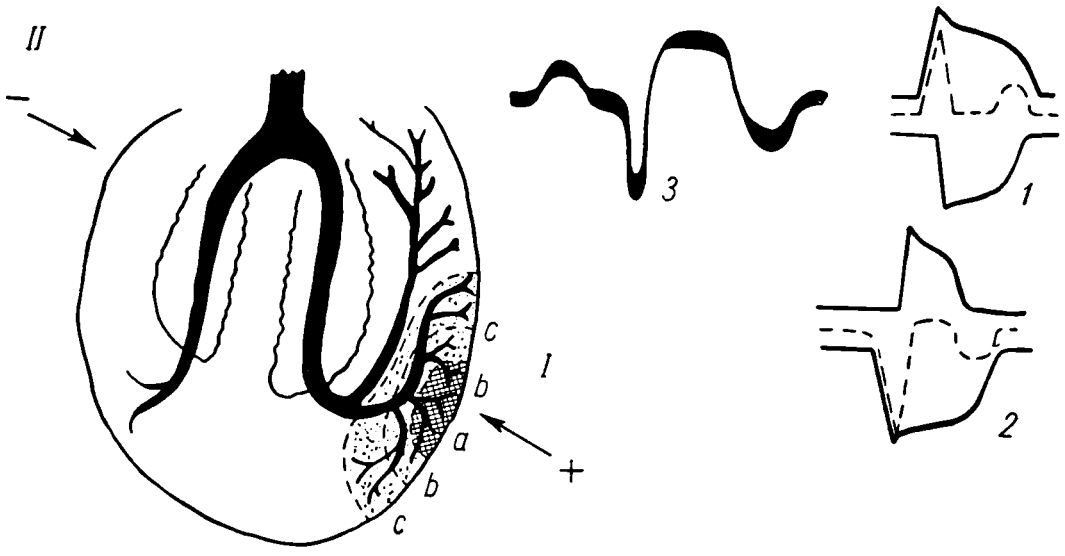

Fig. 1. Correlations between the focus of necrosis and the prenecrotic sphere of the myocardium.

a—area of necrosis; *b-b*—central part of prenecrotic zone; *c-c*—peripheral part of prenecrotic zone. *I* (with arrow +) positive sphere of lead; *II* (with arrow—) negative sphere of lead; *1*—formation of phasic ECG (dotted curve) of two monophasic curves; *2*—same as *1* after onset of infarction in sphere of lead *I*; *3*—deformed ECG (*QS*—dome-shaped *ST*, negative *T*) after its appearance in sphere of lead *I*

trical and contractile activity of the myocardium in a region considerably exceeding in size the necrotic area itself (E.A. Kyandzhuntseva a. M.G. Udelnov).

The region of the myocardium in the sphere of influence of the necrotic focus was named the prenecrotic zone. The correlations between the focus of necrosis and the prenecrotic spheres of the myocardium are schematically presented in Fig. 1.

It was shown in the same experiments that during the action of necrotized tissue on a normal myocardium it is possible to record all the ECG signs of myocardial infarction (E.A. Kyandzhuntseva a. M.G. Udelnov; I.A. Keder-Stepanova a. M.G. Udelnov).

As a result of the action of necrotized tissue on the intact myocardium (acute experiments on frogs, cats and rabbits and chronic experiments on rabbits) we revealed all characteristic ECG changes which may be observed in the acute and the reparative periods both in experimental infarction caused by ligation of the corresponding coronary artery and in infarction under clinical conditions.

Thus, in experiments with the influence of necrotized tissue on a normal myocardium we revealed ECG signs which were usually considered to be "purely ischaemic" (inversion and "giant" *T* wave, displacement of *S-T*) and such changes as were

supposed to become manifest only in cases of a penetrating infarction, extensive scarring and aneurysm (*QS* wave).

The fact that in our experiments all ECG signs of myocardial infarction were determined by the influence of necrotized tissue is no reason to deny the role of ischaemia as a factor responsible for ECG changes. On the contrary, in the initial phase of affection ischaemia is the principal cause of ECG changes. Since the beginning of the necrobiotic process (3rd-5th minute) and particularly after the onset of necrosis ECG changes may be increasingly determined by the active paralysing effect of the necrotic focus upon the surrounding tissues, even if blood supply to the latter is adequate.

As distinct from ischaemia, necrotized tissue produces a prolonged and vigorous effect.

The morphological findings of Karsner and Dwyer obtained by coronary ligation in dogs showed that necrotized tissue disappeared only on the 15th-20th day. Mallory and White also pointed out that elements of necrotized tissue may be discovered in cadavers within three weeks of the onset of infarction. As has been proved by investigations of V.S. Salmanovich another sign of preserved necrotized tissue—an increased potassium level in the perifocal zone during experimental infarction persisted approximately for the same time whereas the potassium level in the ischaemic tissue was reduced.

An analysis of the causes directly responsible for ECG changes during myocardial infarction has shown that the electrical properties of the necrotic focus itself cannot by themselves cause ECG deformation. Only alterations in the bioelectrical activity of the living structures surrounding the necrotized area may be the immediate cause of such changes. Another such cause may be disturbances in the chronotopography of stimulation of the entire ventricular myocardium, which are inevitable during exclusion from activity of a relatively large amount of contractile myocardial tissue and the larger branches of the conducting system. The blocking of the conducting system inevitably occurs if some of its parts are found to be within the sphere of influence of necrotized tissue (V. S. Salmanovich and M. G. Udelnov).

Let us discuss changes in the action potentials which appear in individual myocardial fibres of the prenecrotic zone during the action and after the removal of necrotized tissue (Fig. 2).

In an experiment staged by our collaborators (A.V. Rakova and L.V. Rozenstrauch) quickly developing deep changes were noted in the action potential and the resting potential of the myocardial fibre in the prenecrotic zone. These changes occurred during the action of necrotic tissue and after its removal. The experiments were carried out with the aid of intracellular electrodes.

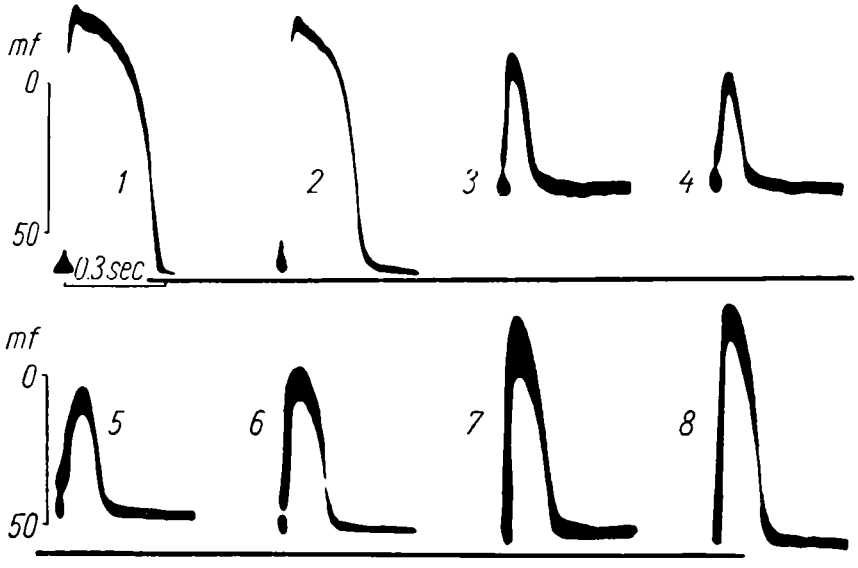

Fig. 2. Change in the action potential of an isolated myocardial fibre lying in the sphere of the prenecrotic zone caused by the influence of necrotized tissue on the myocardium (registered by means of intracellular microelectrode).

1—initial action potential of myocardial fibre; *2*—group of consequently registered action potentials of same fibre at early stage of influence of necrotized tissue; *3-4*—groups of action potentials of same fibre during continued influence of necrotized tissue; upward displacement of initial point of curves points to growing depolarization of fibre membrane (diminution of resting potential); 5-8—restoration of action potentials of fibre after removal of necrotized tissue (terminal phase of restoration is not shown; action potential practically coincides with initial one). Downward displacement of initial point of curves points to restoration of polarization of fibre membrane—increase of resting potential

These changes were concerned with the three major characteristics of the action potential and the state of the myocardial structures of the prenecrotic zone.

First of all, we can see that the restorative process, repolarization, occurred rapidly, with exclusion of a certain very important link. In view of this, there was naturally no restoration of the energy potential of tissue for the next stimulation. Along with a sharp diminution of the amplitude of the action potential, our experiments showed a sharp reduction in the contraction of the trabecular structures of the myocardium, which ultimately became impossible. As a result of uncompleted repolarization the resting potential was reduced, and a very marked depolarization of the cellular membrane came on the scene. At the same time, as has been shown by the investigations of Weidmann, Hoffman and Cranefield, disorders in metabolism and above all in the ion balance are inevitable. Upon removal of the necrotized tissue all these changes gradually disappear.

The above-described changes in the action and resting potentials resemble changes in the myocardium under the influence

of potassium ions. As early as during the experiments carried out jointly with I.A. Keder-Stepanova we proved that potassium excess in the external environment causes tissue depolarization owing to the incompleteness of the repolarization process. This corroborates the fact established at our laboratory (V.S. Salmanovich) that necrotized tissue acts on the myocardium through the medium of potassium ions liberated during the necrobiotic process.

In the same experiments we investigated the influence of atropine on the bioelectrical activity of the myocardial fibre of the prenecrotic zone deeply disorganized by the action of the necrotic tissue. An experiment showed that atropine which is usually administered to exclude parasympathetic nerve influence and improve the blood supply to the heart muscle is a substance effectively acting upon the myocardial structures of the prenecrotic zone (Fig. 3).

As is seen in Fig. 3, atropine aids an early restoration and normalization of the electrical processes in the structures of the prenecrotic zone. Contributing to the normalization of the repolarization-recovery process, atropine tends to normalize the resting potential. It is clear that this is accompanied also by normalization of the functional activity of tissue (in particular, I.A. Keder-Stepanova has found that atropine produces a marked positively inotropic effect which manifests itself irrespective of the nerve influence). These findings explain the fact of diminution of the prenecrotic sphere to extremely narrow limits under the influence of atropinization of the heart, which had been earlier established by I.N. Golovshchikova and I.A. Keder-Stepanova.

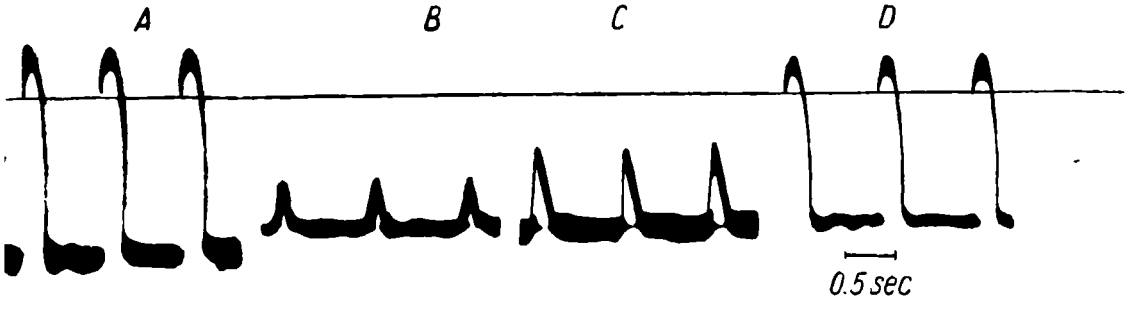

Fig. 3. The influence of atropine on the rate of restoration of the amplitude, form and duration of the resting potential of the myocardial fibre depressed by the influence of necrotized tissue.

A—initial action potentials of fibre; *B*—action potentials in initial phase of restoration; *C*—action potentials after 2 min; *D*—action potentials immediately after addition of atropine to solution (heart preparation after Gramenitsky)

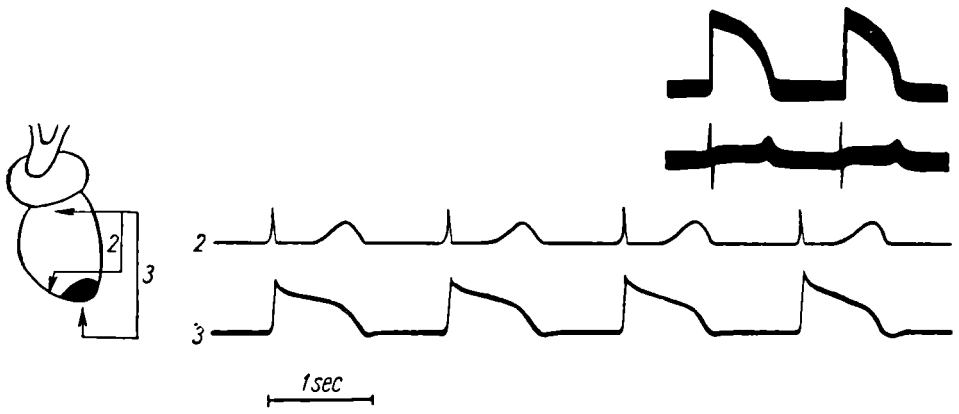

Fig. 4. Coincidence of the monophasic curve of the action potential of an isolated fibre in form and duration with the monophasic electrogram (EG) recorded in the external "unipolar" lead. At the same time, the curves exactly coincide with the phasic EG of the heart recorded synchronously with the monophasic EG (see text)

It is to be supposed that atropine as a superficially active factor, while normalizing the repolarization process, decreases the permeability to potassium, thereby excluding or limiting its depolarizing action on the myocardial elements.

Turning back to the principal subject of our report, we should naturally find out the relationship of the change in the repolarization process, diminution of the amplitudes and reduction of the resting potential in the myocardial structures of the prenecrotic zone to the genesis of the ECG signs of myocardial infarction. It should be noted above all that the monophasic curve of the action potential of an isolated fibre for its general form, correlation in phases and duration is very close to the monophasic curve of the heart electrogram recorded by means of the so-called unipolar external lead.

Fig. 4 presents the correlations between the duration of the monophasic curve of the action potential of an isolated fibre and the phase of the heart electrogram recorded from the heart surface (Hecht). One can see a very close coincidence of the duration of the monophasic potential of the fibre action and the phasic electrogram of the heart (EG in right corner of diagram). During a close coincidence in form and duration of the monophasic curves of the "unipolar" lead (*3*) with the curves of the fibre action potential (upper recording in right corner of diagram) one can see that the monophasic electrogram recorded by means of the external lead (*3*) also coincides with the phasic electrogram (*2*).

It is known that according to the differential theory of formation of the phasic electrogram of the heart and the electrocardio-

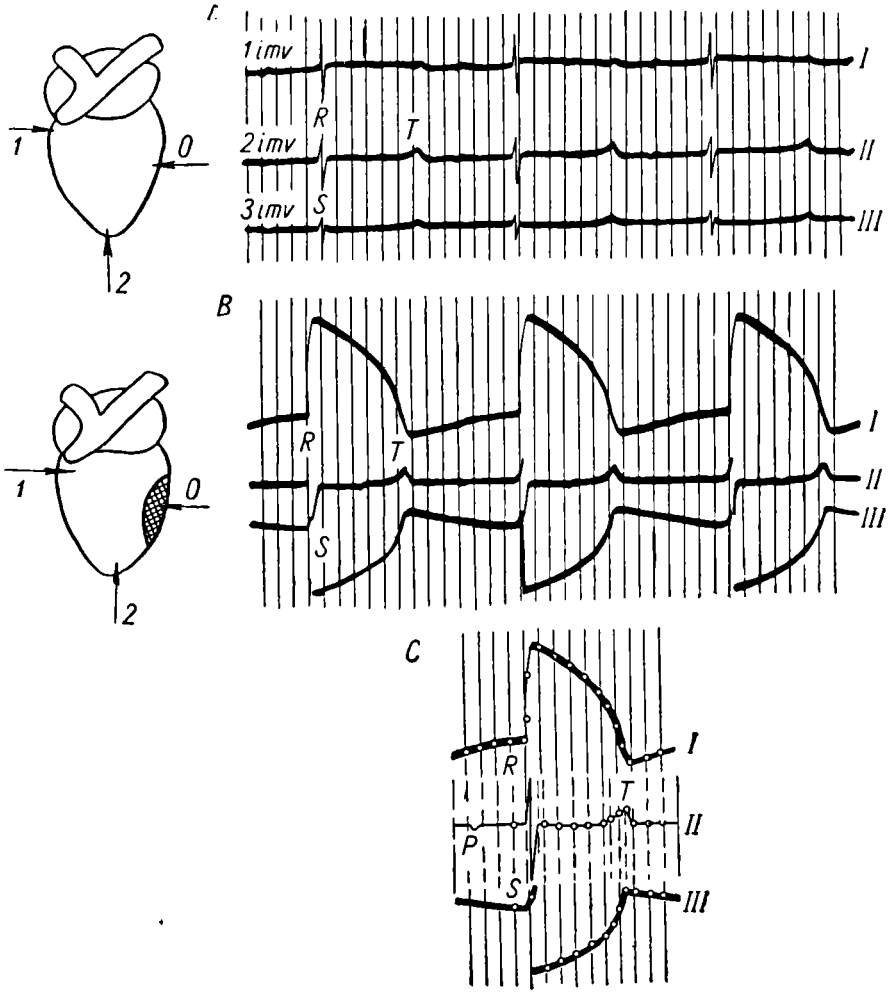

Fig. 5. Electrograms (EG) recorded at the disposition of electrodes indicated in the diagram: *0-1*—lead *I*; *1-2*—lead *II*; *0-2*—lead *III*.

The lower curve shows phasic EG graphically calculated from the monophasic curves of the action potentials from the 1st and 2nd lead zones. One can see complete coincidence of the theoretical (calculation) curve (see middle curve *C*) and EG of the heart taken in lead *II* (middle curve *B*). *A*—EG in bipolar lead; *B*—EG in unipolar leads (upper and lower curves) and bipolar lead (middle curve); *C*—formation of phasic EG from algebraic summation of synchronous points on upper and lower curves

gram they are formed as a result of interference or algebraic summation of the zonal action potentials, which are expressed by monophasic electrograms (Fig. 5).

It is seen in Fig. 5 that as a result of such interference of the zonal action potentials there forms a phasic electrocardiogram fully coinciding with the electrocardiogram recorded in lead *II*.

The electrograms presented in Fig. 6 allow of tracing the change in the phasic electrogram and the electrocardiogram

during a change in the repolarization process in one of the lead zones (Fig. 6).

The dynamics of ECG changes, in particular the "giant" and inverted *T* wave is conditioned here by a disturbance in the repolarization processes in a myocardial portion making one of the lead zones. These changes may be caused either by ischaemia or by the influence of necrotized tissue. None of these causes of disturbance in the reparative-recovery processes can be regarded as universal: in the early stage of affection ischaemia prevails, later (for a longer period)—necrotized tissue.

Disturbance in the repolarization processes and the corresponding ECG deformations may also be due to myocardial intoxication (P.E. Lukomsky, A.N. Berinskaya), local disturbances in the ion turnover, tumours and other causes (D.F. Presnyakov).

To clear up the genesis of *QS* wave we should turn back to the diagram in Fig. 1 showing the correlations between the necrotic focus and the peripheral part of the prenecrotic zone. It will be recalled that the zonal conception (Kisch et al.) of ECG formation specifies that one of the zones is positive, the other negative. It is presumed at the same time that stimulation and electronegativity of the so-called (after Kisch) positive zone, under the accepted conditions of connection to the recording instrument, are recorded as an upward curve, whereas in case of emergence of electronegativity in the so-called negative zone, the curve will be directed downwards from the isoline.

Let us imagine that prior to the onset of infarction zone *I* was positive and zone *II* negative. In this case, upon the appearance of excitation in zone *I* the initial wave of the ventricular complex will naturally be directed upwards. After the onset of myocardial infarction the conditions of ECG formation are changed radically.

In zone *I* only that part of it (*b-b*) can be active which is located along the periphery of the prenecrotic region. And since the branch of the afferent system will at the same time be blocked, excitation in zone *I* will be delayed. As a result of this (see diagram in the right part of Fig. 2) the initial ventricular complex on the electrocardiogram will be shaped as *QS* wave.

Acceleration of repolarization under the influence of necrotized tissue leads to a change in the form and reduction of the length of the curve of the action potentials, which is the very fact that determines the formation of the negative *T* wave.

Taking into account the above-described experimental data, it can be presumed that the bioelectrical causes directly responsible for ECG deformation in myocardial infarction are disturbances in the repolarization processes occurring in the prene-

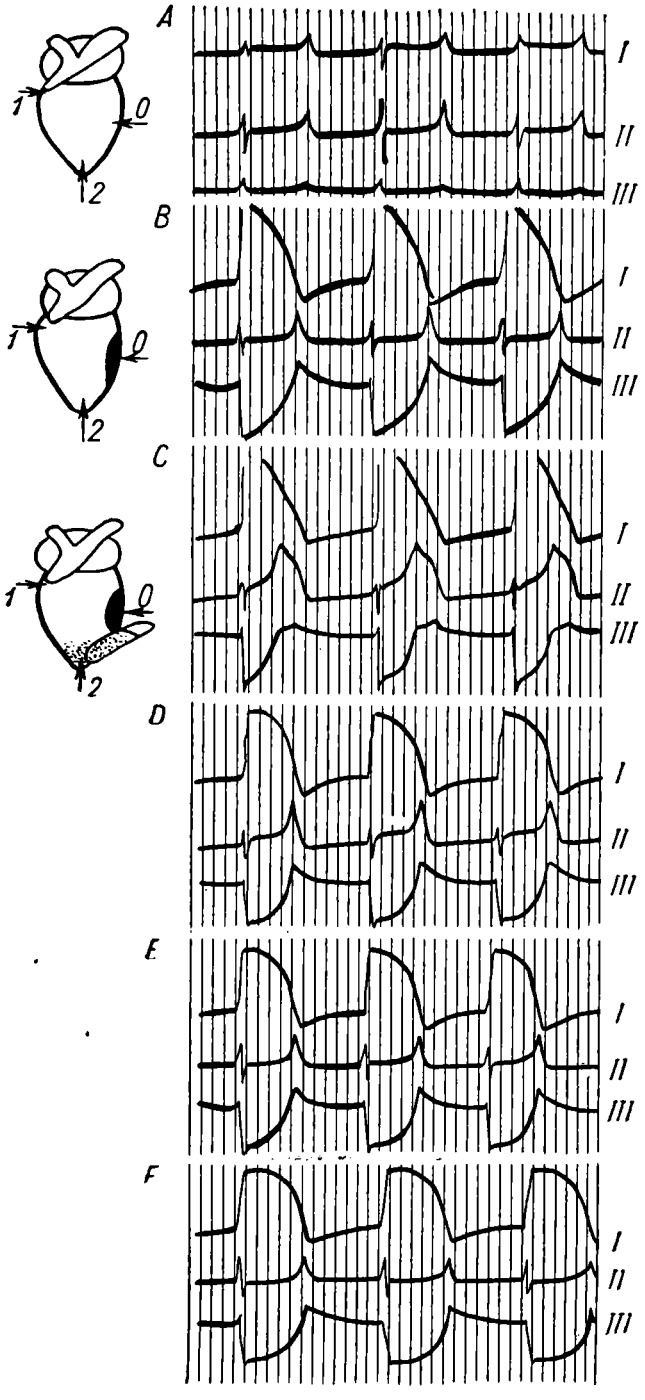

Fig. 6. The influence of changes in the speed of the repolarization process in a portion of the myocardium making one of the lead zones on T wave and ST segment of the heart EG.

Leads: *0-1*—lead *I*; *1-2*—lead *II*; *0-2*—lead *III*;
A—initial EG of three leads; *B*—monophasic EG of leads *I* and *II* and phasic EG of lead *III*; C—same EG after acceleration of repolarization (increase in temperature in zone of lead *II*); a "giant" *T* and upward displacement of segment *S-T* are visible. Acceleration of repolarization is seen on the lower monophasic EG; 3rd phase of repolarization begins earlier and runs a faster course; *D*, *E*, *F*—gradual normalization of monophasic EG of lead *III* and gradual reversal to initial form of phasic EG in lead *II*

crotic zone, decrease in the amplitude of the action potential and the blocking of or injury to the conducting pathways.

In the present report we are unable to present all evidence in favour of the above-described conception of the bioelectrical mechanism responsible for the deformation of ECG curves in myocardial infarction. Presenting only a schematic review of this evidence, we tried to draw the attention of cardiologists to the need for revision of the existing concepts concerning the pathogenesis of the ECG signs of myocardial infarction, which in its turn should introduce certain changes in their diagnostic interpretation.

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THE NEUROGENOUS CAUSES OF MYOCARDIAL ISCHAEMIA IN REPEATED DISTURBANCES OF THE BLOOD SUPPLY TO THE BRAIN

A. V. TRUBETSKOI

As is known, the adaptational regulation of the coronary blood current normally consists in that intensification of the heart's action is accompanied by an increase in the blood supply to the myocardium, and is brought into play by local metabolic mechanisms and neuroreflex stimulations. Myocardial ischaemia may develop during an atherosclerotic affection of the coronary arteries as a result of the narrowing of the lumen and loss of the vasodilating capacity. There is also a view to the effect that myocardial hypoxia may be due to the direct influence of humoral agents during an excessive catecholamine supply to the heart (Raab).

On the other hand, clinicians have good reasons to suppose that myocardial ischaemia may be caused by direct neurogenous constrictive stimulation of the coronary vessels still unaffected by atherosclerosis. For example, G.F. Lang supposes that angina pectoris may be essentially due to a paradoxical reaction of coronary vasoconstriction instead of dilatation. He also spoke of a separate form of angina pectoris "the cause of which is a corresponding functional derangement in the vasomotor-nerve apparatus responsible for the blood supply to the heart muscle."

The present research had for its aim to clear up the conditions of emergence of neurogenous myocardial ischaemia in an experiment with no organic changes in the coronary vessels and no influence of the extracardial humoral factors. The method used in the experiment was humoral isolation of the heart

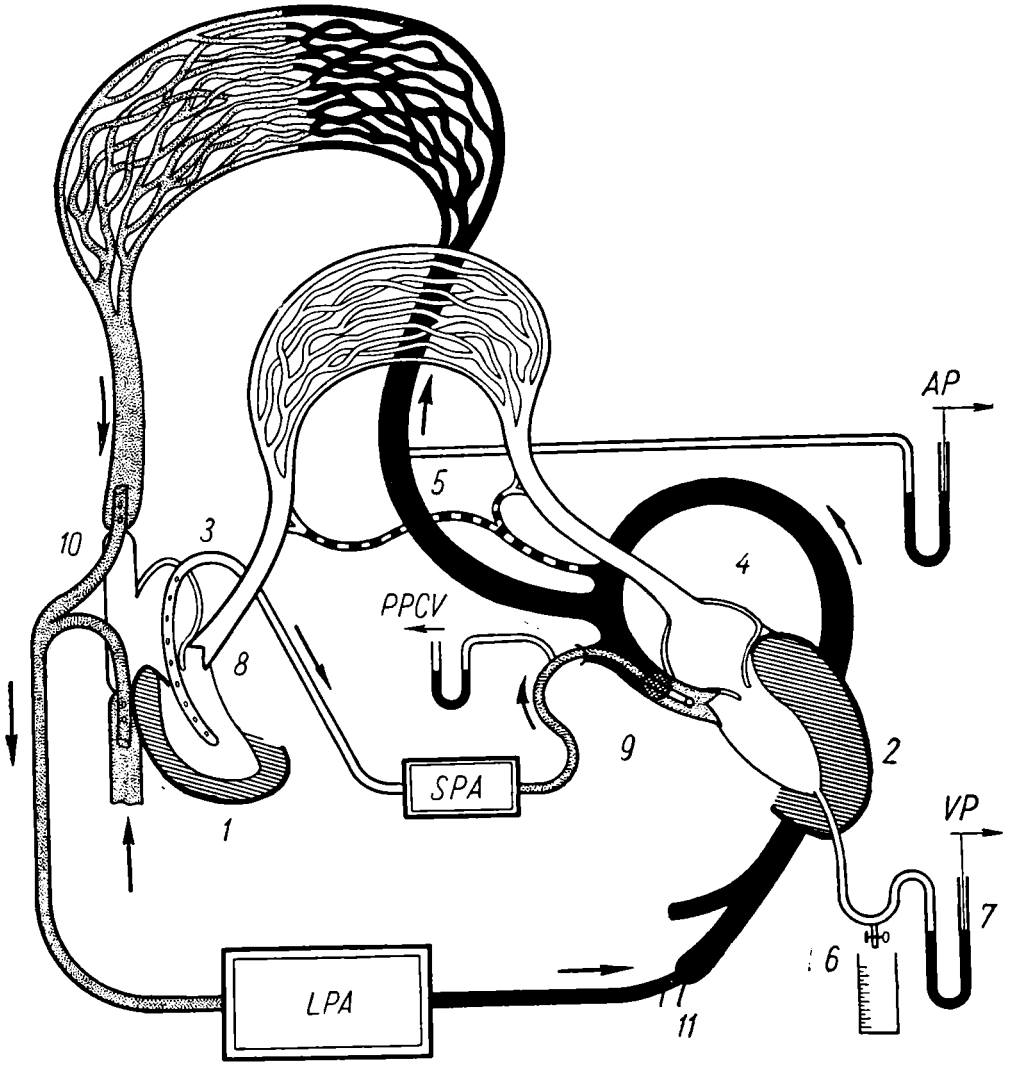

Fig. 1. Sketch diagram of humoral isolation of the heart

1—right ventricle; *2*—left ventricle; *3*—pulmonary artery; *4*—aortic arch; *5*—bronchial arteries; *6*—pipe for draining excessive blood from left ventricle; *7*—manometer for measuring pressure in left ventricle; *8*—catheter for draining coronary venous blood into smaller perfusion apparatus (SPA); 9—catheter for perfusion of coronary vessels; 10—catheters for drainage of venous blood into larger perfusion apparatus (LPA); *11*—catheter for forced delivery

which enabled unobstructed observation of the reaction of this organ to nervous stimulation.

Acute experiments were staged on 14 dogs weighing from 6 to 16 kg. Under the conditions of general combined anaesthesia (0.6 g/kg urethane and 0.04 g/kg urasin on 7 mg/kg morphine basis) the heart was isolated humorally with the aid of two apparatus for extracorporeal circulation using a bubble oxygenator and membrane pumps (A.V. Trubetskoi). The larger perfusion apparatus was used to supply blood to the entire body, the smaller one—only to the heart muscle (Fig. 1). The nerve connections

of the heart remained intact. Under these conditions the heart continued to contract idle. A study was made on the influence produced by repeated stoppage of the larger perfusion apparatus on the rhythm and degree of cardiac contractions, electrocardiogram and coronary blood current. Each successive circulation arrest was effected following complete restoration of the corneal and pupillary reflexes if they had ceased during an arrest. The duration of arrest ranged from 1 to 3 minutes. The larger perfusion apparatus was usually switched on after the appearance of respiratory movements in the animal. In each experiment from 3 to 12 arrests were made. Their number was determined by the state of the animal and the heart. Some arrests were effected in the presence of ventricular fibrillation. The latter was induced with a 5 V alternating current. Defibrillation was induced by brief shocks with a 127 V mains current supplied to the myocardium through spoon-shaped electrodes. The volumetric perfusion velocity was at least 100 ml/kg/min. The coronary blood current was 80-120 ml per 100 g of heart weight. The output of the smaller pump and the resistance of the coronary vessels determined the degree of the coronary perfusion pressure.

During the experiment, the pneumogram, the systemic arterial pressure, maintained by the larger perfusion apparatus, the perfusion pressure in the coronary vessels produced by the smaller perfusion apparatus and the pressure in the left ventricle were recorded. The latter pressure was used for judgement on the intensity of cardiac contractions. The pressure was recorded on a "Cardiovar-VI" apparatus with the aid of "Barovar" electromanometers. Simultaneously, the pressure was recorded on a kymograph chart by means of mercury manometers. The pressure in the vena cava superior was registered with a water manometer. The rate of the venous blood current was measured with a volume speed meter. The ECG was recorded in standard leads II and III.

The arrest of the blood current in the general circulation stimulated the activity of a humorally isolated heart and the coronary tone. In the majority of experiments, the first arrest of the blood current caused a decrease in the heart beat rate, and often arrhythmia and extrasystole. For example, in one of the experiments the heart-beat rate dropped from 125 to 40 per min. Sometimes, the heart-beat rate at first increased somewhat and then became sharply reduced. Cases of bradycardia or an unchanged rhythm were invariably accompanied by disturbances in the conductivity and excitability of the myocardium. In the majority of cases, the subsequent arrests of the blood current were followed within the first ten seconds only by an increase in the heart-beat rate which attained a maximum of 200 per min in 20-50 seconds' time (Fig. 2).

With the resumption of the blood current all these changes disappeared within the first several scores of seconds. An analysis of ECG data showed that during the arrest of the blood current in the general circulation heterotropic foci of excitation formed in the humorally isolated heart with a disturbance in conductivity to the degree of block of a variable kind. In the experiments in which a large number of repeated arrests of the

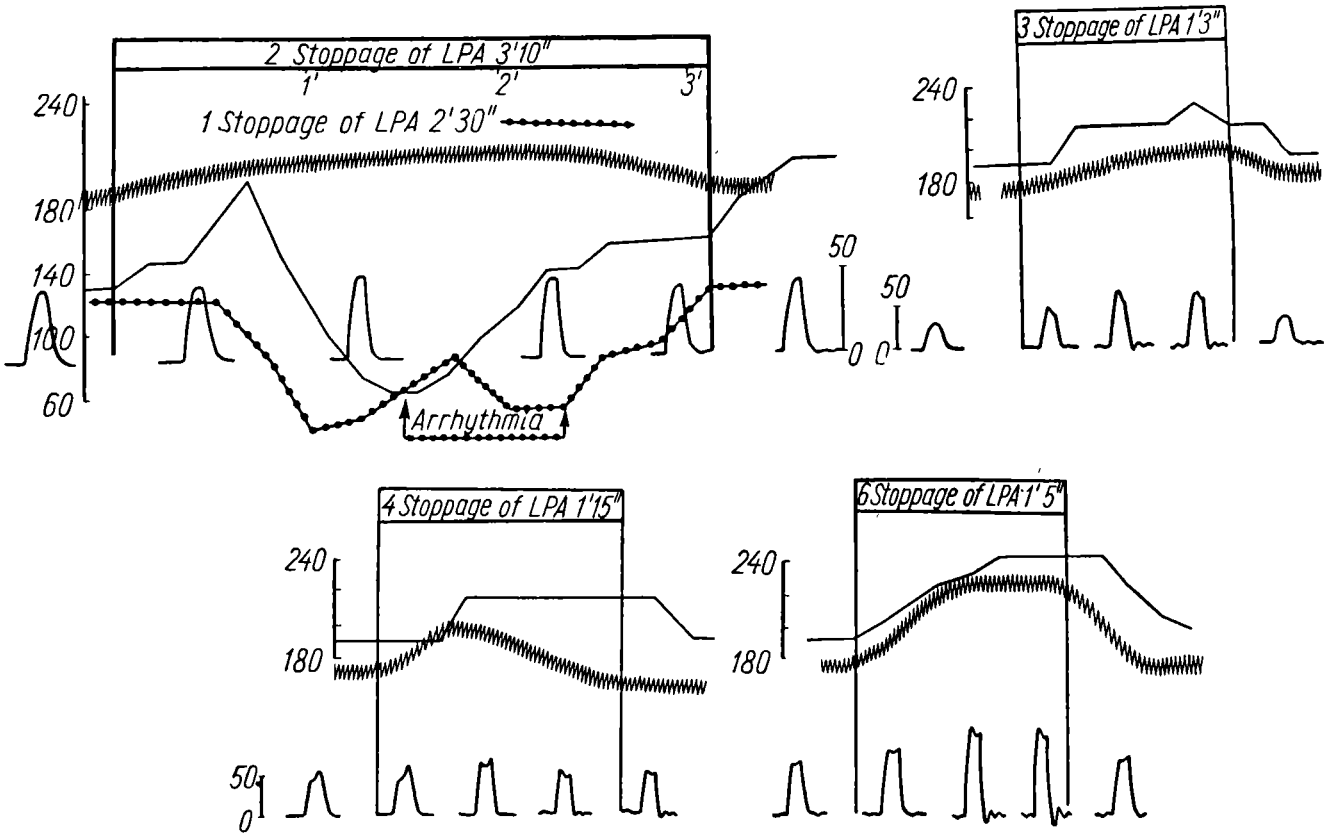

Fig. 2. Increase of the reaction of a humorally isolated heart in the course of six repeated arrests of the blood current in the general circulation.

Broken line—general resistance of coronary vessels, continuous line—heart beat rate (line with crosses indicates change of heart beat rate during first arrest), below—form of intraventricular pressure curve

blood current (10-12) were undertaken they were followed by displacement of the *S-T* interval, appearance of the dome-shaped *T* wave, increase in the latter's voltage. A macroscopic examination of the heart in such experiments revealed tiny haemorrhages under the endocardium, epicardium or in the thickness of the myocardium.

In addition to a change in the conductivity, excitability and rhythm, the arrest of the blood current caused intensification of cardiac contractions. In certain cases, it reached 250 per cent of the initial degree of the intraventricular pressure assumed to be equal to 100 per cent. The maximum increase in the intensity of cardiac contractions occurred on the average 40-60 seconds after the beginning of cessation of the general circulation. This was followed by a decrease in the intensity of cardiac contractions. Just as in relation to the increase in the heart-beat rate the intensity of cardiac contractions began to grow within the first ten seconds of cessation of the general circulation and reached a maximum when the heart-beat rate had already started to decline. After attaining a maximum, the intensity of cardiac contractions decreased although the general circulation was still arrested. Sometimes, the starting of the larger perfusion apparatus was in 20-60 sec followed by a repeated increase in the intensity of cardiac contractions though not so considerable as it had been upon the stoppage.

During the experiment, several repeated simultaneous arrests of the general circulation were undertaken, which in the majority of cases evoked each time an ever stronger reaction of the heart. An increase in the reaction of a humorally isolated heart could be observed also in those cases where the duration of subsequent arrests was shorter than that of the first ones (Fig. 2).

In addition to the above-described changes in the heart's action the arrest of the general circulation caused an increased resistance of the coronary arteries, which was registered by two indices: elevation of the perfusion pressure and reduction of the coronary venous blood drainage. An increase of resistance could be observed both during a considerable intensification of cardiac contractions and during comparatively weak positive inotropic reactions associated with bradycardia. In the latter case, comparison of the curve of perfusion pressure in the coronary arteries with the intraventricular pressure curve recorded by means of electromanometers showed that the perfusion pressure level had risen during the diastole (Fig. 3). Such an increase in pressure during the diastolic relaxation of the myocardium could evidence nothing but an increase in the tone of the coronary vessels as a result of direct constrictive nervous stimulations.

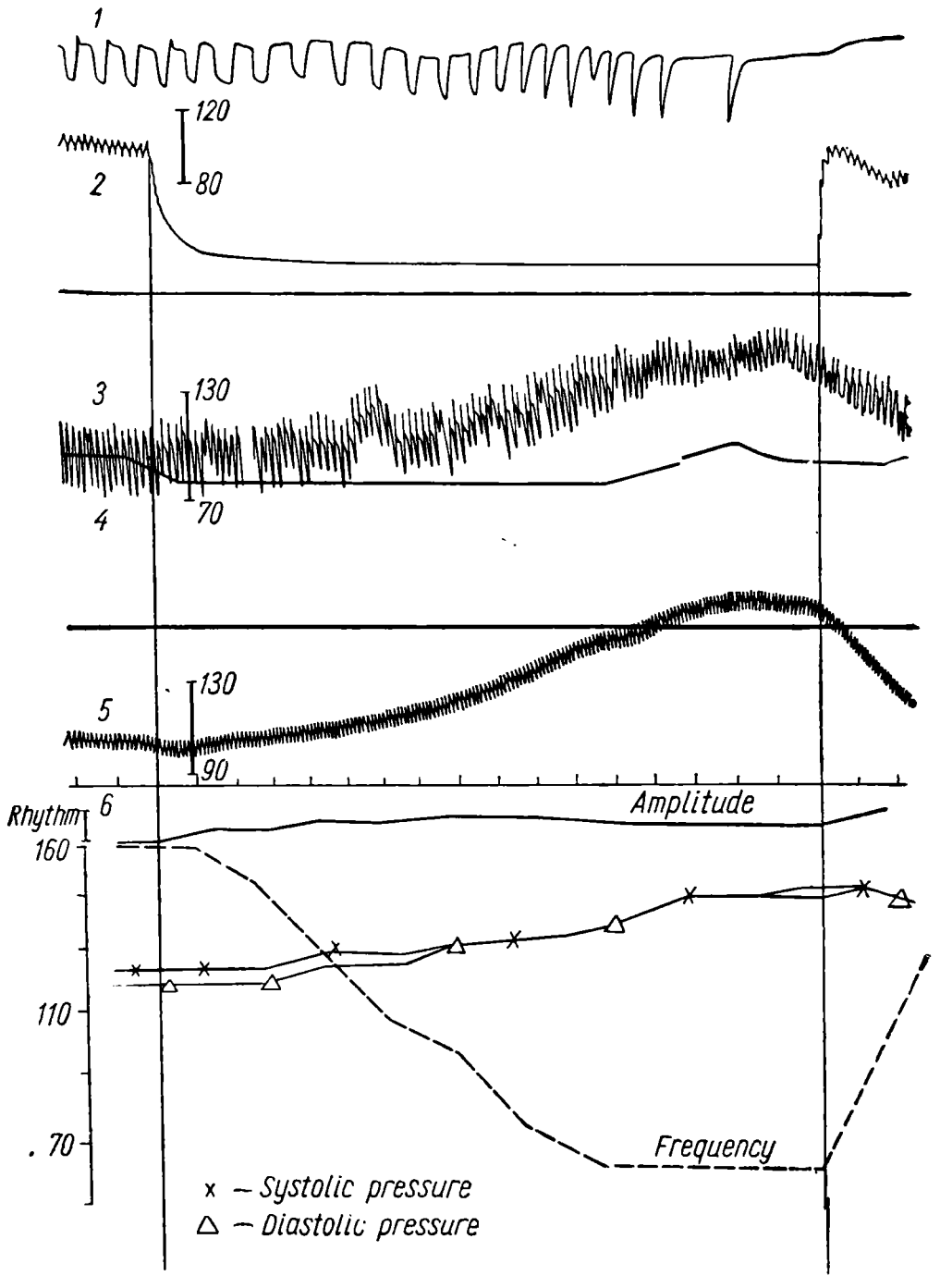

Fig. 3. The influence of arresting the blood current in the general circulation for 1 min 25 sec on the tone of the coronary vessels, the heart's action and resistogram of the leg vessels.

From above downwards: 1—respiratory movements; 2—systemic arterial pressure; 3—perfusion pressure in coronary arteries; 4—systemic venous pressure; 5—resistogram of leg vessels; 6—time mark of 5 sec. The four lower curves show the results of processing electromanometer readings. Curves 2, 3 and 5 have been recorded by mercury manometers

The independence of the neurogenous coronary vasoconstriction was found also in those cases where the blood current was arrested during ventricular fibrillation, i.e., in the complete absence of synchronous rhythmic contractions of the myocardium as a whole.

If arrest of the blood current in the general circulation caused an increase in the intensity and rate of the heart beats, the resistogram of the coronary vessels showed a summary change in their tone both during the diastole owing to the above-mentioned nervous stimulation and during the systole as a result of the increased mechanical compression of the vessels by the heart muscle. No coronary vasodilatation in the period of diastole or at least stability of their tone was observed in these cases.

In four experiments made with the aid of a separate perfusion pump—resistograph, changes were recorded in the vascular resistance of the left leg in the dog during the arrest of the general circulation. At the same time, an increase was noted in the resistance of the vascular bed in the leg. One of such examples is presented in Fig. 3. One could note a great parallelism existing between changes in the tone of the coronary vessels and that of the vessels of the leg. The reaction of the leg vessels to each successive arrest was increasingly intense.

It is widely known that hypoxia and anoxia are a powerful factor stimulating nerve structures, in particular the centers regulating cardiovascular activity (Brooks, Alexander, Rushmer, Schaefer). These authors registered the excitation of the centers in oxygen insufficiency both for the electrophysiological and haemodynamic indices. Gömöri and his collaborators, during a selective hypoxia of the dog head, observed ECG changes which they ascribed to ischaemia. In view of these findings, we employed such a method which enabled us to arrest the general circulation without disturbing the blood supply to the heart. During this procedure, hypoxia (anoxia) developed in the entire body, including the central nervous system, but excepting the heart muscle. In spite of certain shortcomings of this method (idle contractions of the heart, complete blood drainage from the lungs, open thorax, etc.) the nervous influences on the heart which took place under these conditions were more natural and adequate than in the case of electric stimulation of a certain nerve.

The temporary arrest of the general circulation caused changes in the heart's action, the coronary resistance, which were also reflected on the electrocardiogram. Because the coronary blood current was sustained from a separate source, while the heart was humorally isolated, the stoppage of the larger perfusion apparatus could by no means have an effect on the blood supply and the heart's action. Therefore, the changes observed

could not be other than neurogenous. Judging by the reaction of the heart, the nervous stimulations were characterized by an increase of effect during each successive stoppage in spite of the same and in certain cases even smaller degree of stimulation. If the above-mentioned increase in the intensity and rate of contractions should be regarded as an adaptation reaction because they sustain the blood current in case of intact circulation, the observed increase in the coronary bed resistance should be described as a pathological reaction increasing the discrepancy between the oxygen requirements of the myocardium and the oxygen supply. The increase in the resistance of the coronary vessels during diastole is evidence of the presence of a neurogenous constriction. The conditions of humoral isolation permit the assertion that this constriction was the result of changes in the extracardial nervous stimulations and could not be contingent on the state of the heart itself or its vascular network. Apparently, hypoxia (anoxia) of the central nervous system had led to a disturbance of its adaptational regulatory activity which gave rise to pathological reflex nerve influences on the coronary vessels.

In addition to the neurogenous increase in the tone of the coronary vessels their resistance also grew as a result of intensified heart action in experiments. Salisbury and his collaborators and Cross found that the coronary blood current is in a linear relationship with the difference between the pressure at the ostium of the coronary arteries and the intraventricular pressure, which was named as the coronary propulsion pressure. This relationship was preserved both during the action of the left ventricle at normal load and during its exclusion from the blood circulation. According to these authors, in hypoxaemia the correlation between the coronary propulsion pressure and the coronary blood current remained unchanged, although the absolute volume of the coronary blood had increased. Our findings corroborate the conclusions of Salisbury and his collaborators concerning the role of the mechanical factor in the regulation of the coronary blood current.

All this is good reason to presume that in cases of neurogenous coronary vasoconstriction the intensification of the heart's action was combined with failure in coronary dilatation, producing a sharply increasing discrepancy between the increased oxygen requirements and oxygen supply.

As has been pointed out above, in the course of repeated arrests of the general circulation, increasingly manifest reactions occurred in the humorally isolated heart. At the same time, the reaction already depended not so much on the duration of arrest as on its ordinal number. This was evidence of the growth of sensitization of the nerve apparatuses regulating the heart's

action. Interesting findings were made in the experiments performed by Busmengo and Paolucci, Gammarata and Cardaci, Varma and Melwille. They used various modifications of experiments in which animals (rabbits, dogs) were put into altitude chambers with a reduced partial oxygen pressure for 20-40 minutes daily during a period of 15-120 days. Beginning from the 6th-10th session, the authors observed that the same degree of hypoxia began to cause a sharper increase in the heart-beat rate and a change in the electrocardiogram (elevation of the *ST* interval and increase in *T* wave voltage). Every next time, the heart reaction in response to hypoxia was faster. Upon completion of a series of experiments dissection revealed all signs of myocardial ischaemia. Analogous data, although referring to atherosclerotic rabbits, were obtained by N.N. Kipshidze. According to evidence by E.V. Leonova, the "elevation" of animals within an altitude chamber to a height of 4,000-5,000 m caused in them failure of the compensatory reactions preliminarily developed for compression of the carotid arteries. In addition, one could observe a reduction of the maximum altitude and development of oxygen deficiency within a shorter time.

In addition to the above-described methods used by other authors, we induced a hypoxic state by means of arresting the general circulation. Diminution of the peripheral pressure was naturally followed by a change in afferent impulse emission. It can be presumed that this contributed to the disorganization of the integrative function of the central nervous system affected by hypoxia. In this connection, mention should be made of S.I. Baluyev's data to the effect that in the reaction of the central nervous system to the humoral agent a substantial role is played by signals from the peripheral chemoreceptors stimulated by this agent.

In spite of the considerable methodological differences between the experiments of the above-mentioned authors and our experiments, comparison of the findings obtained permits the conclusion that during general hypoxia the predominant role in the heart reaction is played by the neuroreflex rather than humoral influences. If multiply repeated, these influences, along with intensification of the heart's action, begin to cause coronary vasoconstriction.

There are observations on the state of the cardiovascular system in man during acute and chronic hypoxia. For example, Simonson, while drawing a comparison between the reactions of vessels to hypoxia noted that changes in elderly persons were more severe than in young ones. This difference was ascribed by the author to a reduction in the sensitivity threshold of the vasomotor center as a result of atherosclerotic cerebral disturbances.

From the findings of E.V. Andrushchenko (1958) made on 127 patients with hypertension it follows that the greater the degree of hypoxia the more evident the signs of the concomitant disease—angina pectoris. The treatment of these patients leading to abatement of hypoxia also eliminated the symptoms of coronary insufficiency.

Comparison of the experimental data and clinical observations obtained, it is true, under different conditions suggests the idea that the chronic state of hypoxia often occurring in elderly persons due to a variety of causes, in particular during hypertension (F.Y. Primak) may contribute to a disturbance of the adaptational regulation of the cardiovascular system carried out by the central nervous system. At the same time, inadequately increased contractions of the heart occur in response to a certain stimulus and are accompanied by coronary stenosis, which, naturally, leads to acute myocardial ischaemia.

Under these conditions, hypoxaemia and catecholamines, acting directly on the heart muscle, increase the discrepancy between the oxygen requirements of and oxygen supply to the myocardium.

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CHANGE OF AFFERENT SIGNALIZATION FROM THE HEART IN EXPERIMENTAL MYOCARDIAL INFARCTION

F. P. YASINOVSKAYA

It is known that in myocardial infarction considerable changes occur in the activity of the entire cardiovascular system (reduction of blood pressure, redistribution of the vascular tone, change of the capillary circulation, etc.). There is a disturbance in respiration, the digestive tract, and a change in the neuropsychic sphere. In time, the deranged functions are compensated and restored. In severe cases, however, decompensation may develop as well as such formidable complications as ventricular fibrillation and collapse which are the principal causes of death in myocardial infarction.

Numerous clinical and experimental findings point to an important role played in all mentioned phenomena not only by the presence of a lesion focus as such and the associated haemodynamic disorders but also by the pathological reflexes from the heart (G.F. Lang).

The varied and extensive system of cardiac receptors (B.I. Lavrentyev, E.K. Plechkova, A.Y. Khabarova, et al.) is the morphological foundation of an uninterrupted supply of accurate information on the heart's action to the central nervous system. Bainbridge's, Bezold-Jarisch's and other reflexes regulating the blood pressure level, cardiac rhythm and respiration are widely known. The afferent path of these reflexes begins from the heart.

In the past few years, the heart as a reflexogenic zone has been comprehensively studied by B.S. Kulayev, a researcher at the physiological laboratory of the Institute of Therapy (1954-1962) who has shown the reflex stimulation of the heart on the

blood pressure and a change in the peripheral resistance of various vessel regions. B.S. Kulayev showed also a change in these reflexes during compression of the coronary artery, which clearly indicates the origination of deep changes in the receptor activity.

At the present time, one of the methods used for the study of the reflex activity is registration of the electrical activity of the afferent, central and efferent formations.

Even the first investigations of the afferent impulses from the cardiac nerves in the total electrical activity of various fibres revealed impulse volleys in the cardiac rhythm (Aman and Schaefer; Jarisch and Zotterman). These volleys are the result of stimulation of the mechanoreceptors embedded in the walls of the cardiac atria and ventricles. A change in the cardiac haemodynamics entails a change in the duration and frequency of a volley.

Later, Paintal, Langrer and others showed on the example of isolated afferent fibres a correspondence between a dosed filling of the cardiac cavities and the quantitative gradation of the impulse frequency in a volley. Various substances (acetylcholine, KCI, veratrine, serotonine, to mention a few) cause an increase in the number and frequency of impulses in a volley passing through an afferent fibre.

Afferent impulses from the heart are conveyed to the central nervous system. Hellner and Baumgarten observed impulse volleys from the cardiac receptors in the sensory cells of the vagus nerve nuclei in the medulla oblongata. We demonstrated in 1958 and 1961 (by the method of registration of primary impulses) the arrival of afferent signals from the heart at definite zones of the brain cortex.

In its turn, a change in the state of the nerve centres affects the efferent impulses conveyed to the heart and vessels, thereby influencing the functions of the entire cardiovascular system (B.S. Kulayev).

All these data indicate that the great adaptational capacity of the cardiovascular system, its distinct interrelation with the activity of the entire body are produced and controlled under the influence of constant and accurate signals conveyed from the heart.

The question is whether the afferent impulses from the heart change in experimental myocardial infarction.

Kaindl and his collaborators and Stupler found that the afferent activity of the cardiac nerves is intensified by compression of the coronary artery. E.G. Petrova observed in similar experiments a decrease or disappearance of the afferent impulses on the "amplifying" nerve whereas on the lower cardiac branch the

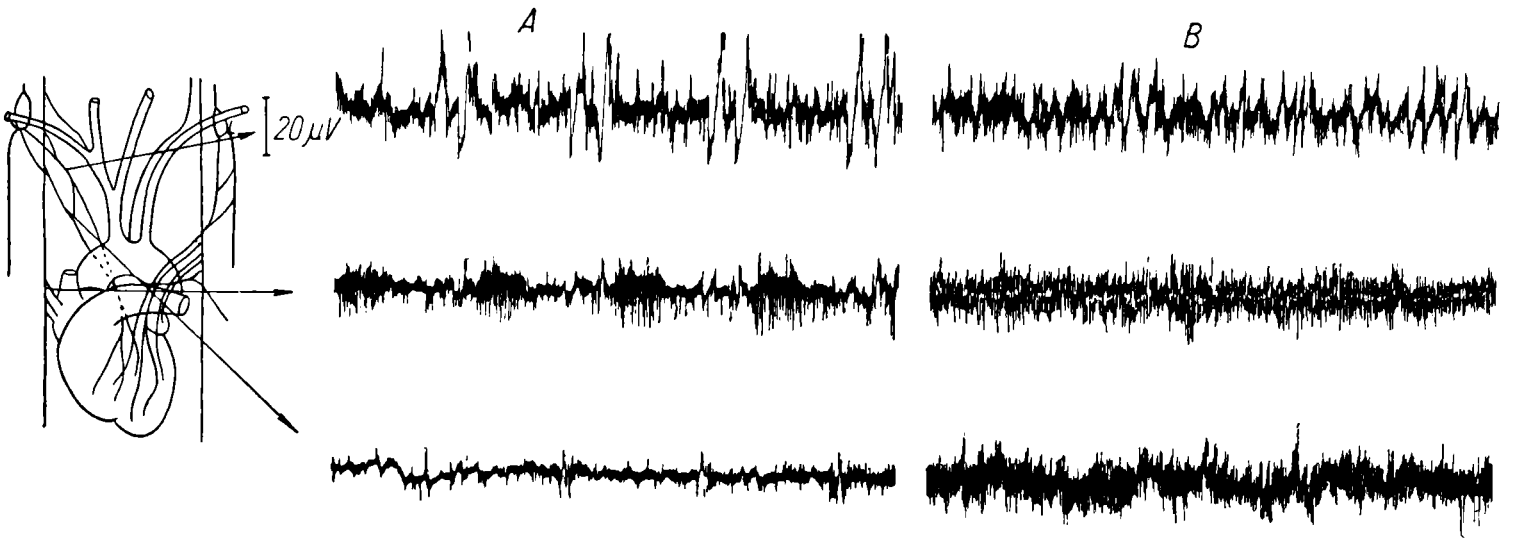

Fig. 1. Afferent impulse emission in various branches of the cardiac nerves of the cat under normal conditions (*A*), after compression of the right descending artery (*B*).

Upper curve—neurogram of right, "amplifying" cardiac branch; middle curve—neurogram of branch leading to right atrium; lower curve —neurogram of right middle cardiac branch

impulses either became more intense or remained unchanged altogether. Literary data and our experiments on cats show that as a result of ligation of the coronary vessel and emergence of a focus in the heart the afferent impulses are, as a rule, altered. The following findings were made during the various changes of the afferent activity of the cardiac nerves:

1. Outbreak of impulses as a result of stimulation of the mechanoreceptors of the ligated coronary artery.

2. Decrease of impulse activity to the point of extinction (mainly on "amplifying" nerve). Such a reaction, in B.S. Kulayev's view (see B.S. Kulayev's article in the present collection, p. 159), may be accounted for by a temporary deafferentiation of the myocardium as a result of ischaemia.

3. (a) Intensification of "quick" impulses (reflected activity of mechanoreceptors) will be discussed below.

(b) Intensification of "slow" impulses is apparently the result of stimulation of the cardiac chemoreceptors (V.N. Chernigovsky, B.S. Kulayev, et al.). Another evidence in favour of this point of view is our finding that intensification of slow impulses is conjugate with the appearance of a "coronary" *T* on the electrocardiogram.

An example of a change in the afferent activity of whole coronary branches is given in Fig. 1. The upper curve (Fig. 1*A*) indicates the initial activity of the principal, "amplifying" cardiac branch; here "quick" and "slow" impulses are registered. The middle curve indicates the activity of the small branch leading to the atrium. It clearly shows the volleys of impulses reflecting the activity of the atrial mechanoreceptors. The lower curve indicates the activity of the median branch (see diagram in Fig. 1). After occlusion of the coronary artery (Fig. 1*B*) one can see a considerable intensification of the afferent activity in all of the three branches. On the "amplifying" cardiac branch the "slow" impulses are increased. On the atrial branch the volleys give place to a continuous flux of impulses.

These curves reflect a disturbance in the frequency and amplitude correlations in the afferent signal system during compression of the coronary artery and emergence of an ischaemic focus. An especially serious reconstruction is visible in the curve showing volleys converted into a continuous flux of impulses. The variety of changes in the afferent impulses and difficulties involved in their analysis on a whole trunk reflecting the activity of hundreds of different receptors just as the variations in the heart innervation and arrangement of the coronary arteries have induced us to undertake a study of the activity of the fibres leading from isolated receptors or small groups of receptors.

In experiments on cats we split cardiac nerves and found an

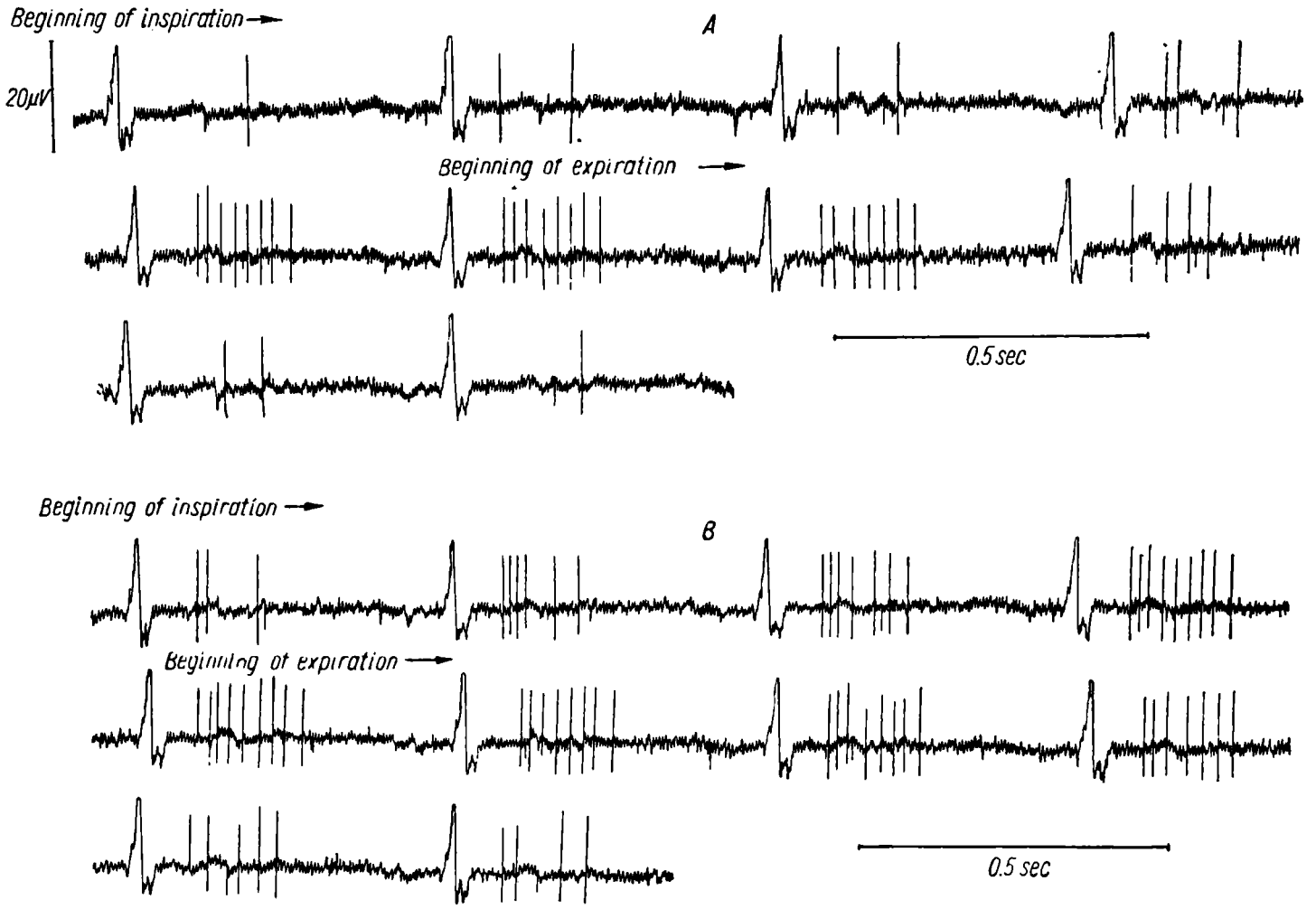

Fig. 2. Afferent impulse emission in an isolated fibre from the mechanoreceptor of the left atrium exposed from the left inferior cardiac branch. ECG imposed on the neurogram because of the proximity of lead electrodes to the heart.

A—volley activity of receptor under normal conditions; perpendicular lines indicate impulses in volley; *B*—activity of same receptor after compression of r. circumflexus and formation of ischaemic focus in left ventricle. The number of impulses per volley is increasing compared to that under normal conditions.

isolatedly functioning fibre of the cardiac mechanoreceptor. The electrical activity of the fibre and the electrocardiogram were recorded by means of a "Disa" electromyograph.

The activity of an isolated mechanoreceptor fibre is characterized by impulse volleys of one and the same amplitude in the cardiac rhythm. The characteristics of the volley (number and frequency) are different in the atrial and ventricular receptors. A change in the volley characteristics with a change in the blood influx during a respiratory cycle, just as the reaction to compression of the venae cavae, the pulmonary veins, the aorta, etc., makes it possible to determine the cardiac cavity to which the receptor belongs. The experiment was rounded off with incision of the heart and identification of the receptor.

Fig. 2*A* presents a neurogram of the fibre of the left inferior cardiac branch from an isolated receptor located in the left atrium (electrocardiogram is superimposed on the neurogram because of the proximity of the lead electrodes to the heart). This receptor responds with one impulse volley synchronously with the atrial diastole. The stimulation of the receptor takes place in consequence of the blood influx into the atrium. During the respiratory cycle the number of impulses in the volley grows from 1 (in expiration) to 8 (in inspiration) as a result of an increased blood supply to the atrium, and then declines to 1, reflecting the minimal influx during expiration.

During compression of the r. circumflexus there forms an extensive focus of ischaemia in the antero-lateral and partly in the posterior wall of the left ventricle with atrial ischaemia. The receptor activity is changed (Fig. 2*B*). Now the minimum number of impulses (in expiration) is equal to 3; when the inspiration begins, the blood supply to the atrium increases, and the growth of the number of impulses is steeper, reaching a maximum of 9 in the volley (at the peak of inspiration), which is followed by a reduction to 4 impulses (in expiration).

This curve shows that in compression of the coronary artery the mechanoreceptor responds with a greater number of impulses in the volley as compared to the corresponding (in the respiratory cycle) volley under normal conditions.

In the following experiment the fibres were split in the cervical part of the vagus nerve after Paintal's method modified by A.P. Gilev, which allowed registration of the activity of the afferent fibres making part of the vagus nerve during natural respiration, i.e., preservation of normal reflex relationships.

The volleys in the fibres leading from the heart were identified by their coincidence with an electrocardiogram in time. The heart was approached by Drinker-Kulayev's method as follows: during artificial respiration the pericardial cavity was

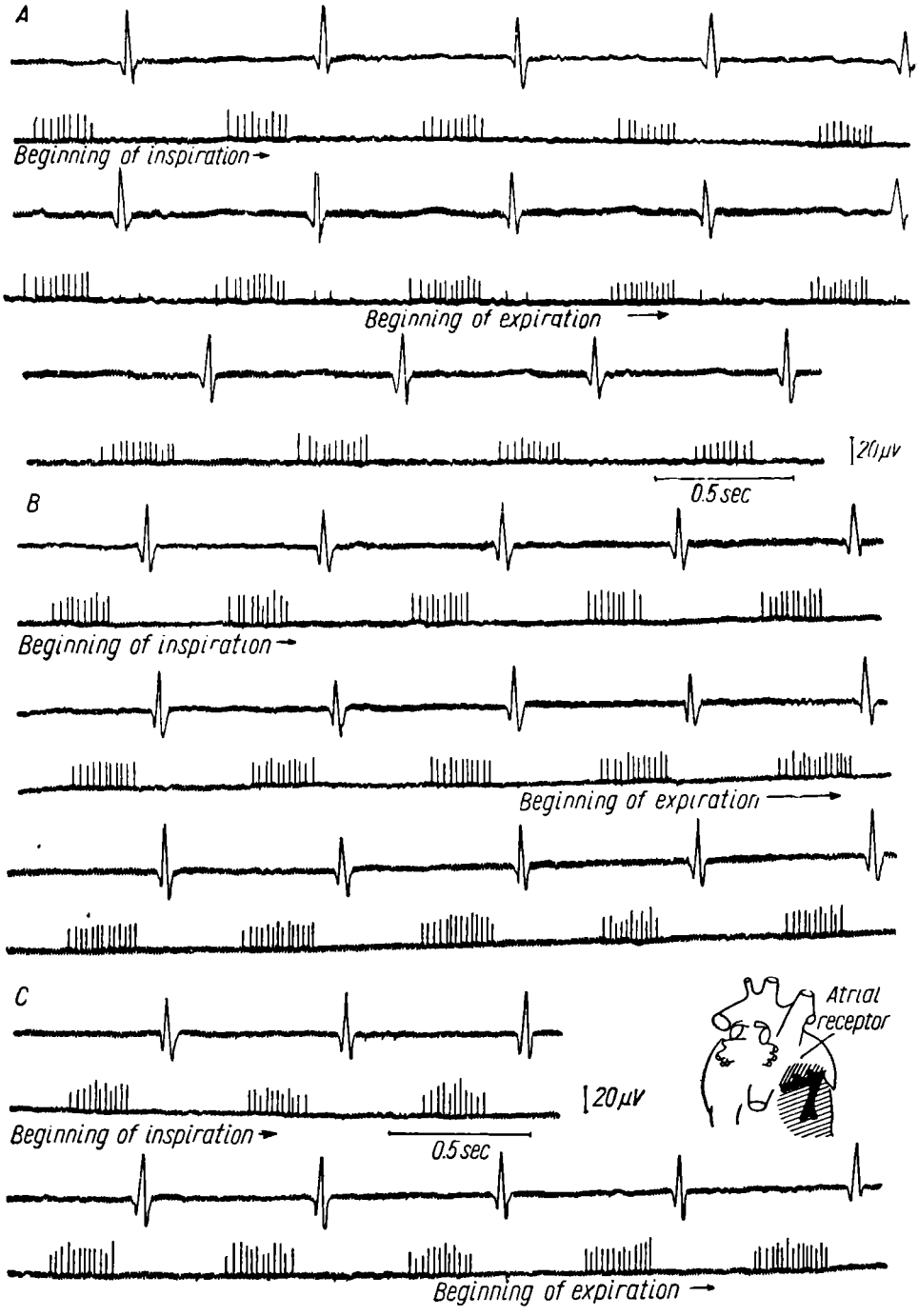

Fig. 3. Afferent impulse emission in an isolated fibre exposed from the right cervical vagus nerve. The mechanoreceptor is located in the posterior wall of the right atrium.

A—volley activity of receptor under normal conditions during change of venous blood influx in respiratory cycle;
B—activity of same receptor 30 sec after compression of right descending coronary artery and formation of ischaemic focus in right ventricle with ischaemia of atrium; *C*—activity of same receptor one minute after compression of coronary artery

opened, and the pericardial margins were sutured to the edges of the skin wound. Upon restoration of the hermetic condition of the pleural cavity the natural respiration returned to normal. In addition to the cardiac fibres the vagus nerve conveys the afferent fibres from the lungs (discharge coincides with inspiration or expiration) and fibres from the receptors of the gastrointestinal tract; their activity is irregular.

Figs. 3 and 5 (upper chart) show the activity of the fibre from an isolated mechanoreceptor of the right atrium with one volley of impulses coinciding with the diastole in time. The diagram shows the relationship of the change in the number of impulses in the mechanoreceptor volley to a change in the venous duct (during the respiratory cycle). Normally, the number of impulses in a volley grows from 7 at a minimum venous influx during expiration to 12 at a maximum venous influx at the peak of inspiration. These characteristics remain almost unchanged for a long time while the animal is in a good condition. During ligation of the right coronary artery (see chart in Fig. 3) one can observe the above-mentioned regularity: the minimum number of impulses in a volley is 10, maximum 14, each volley containing a larger number of impulses than the corresponding normal volley (in the respiratory cycle). There is also a change in the volley configuration; the course of increase and decrease in the number of impulses per volley becomes irregular and ununiform.

Similar relationships are observed also in records of the volley in fibres from a group of receptors. Fig. 4 shows impulse volleys in a group of fibres leading from three receptors. Fig. 5 (lower chart) shows the relation of the number of impulses in the summary volleys (three mechanoreceptors functioning) to a change in the venous influx associated with the respiratory cycle. These receptors respond to each heart contraction with two volleys of impulses. Each volley corresponds to one half of the column on the chart. The left half shows the number of impulses in the summary volley responding to the atrial systole. The right half indicates the number of impulses in the summary volley corresponding to the diastole.

As is seen in curve A (Fig. 4) and the lower chart (Fig. 5), normally the receptors are activated one by one along with an increase in the venous influx during a respiratory cycle, the number of impulses in the volley of each receptor increasing simultaneously. The number of impulses in the diastolic volley grows faster than in the systolic one. At a minimum venous influx only one atrial receptor operates (1 impulse in systolic, 4 in diastolic volley), whereupon the 2nd and then 3rd receptors are brought into play. The maximum number of impulses at the largest

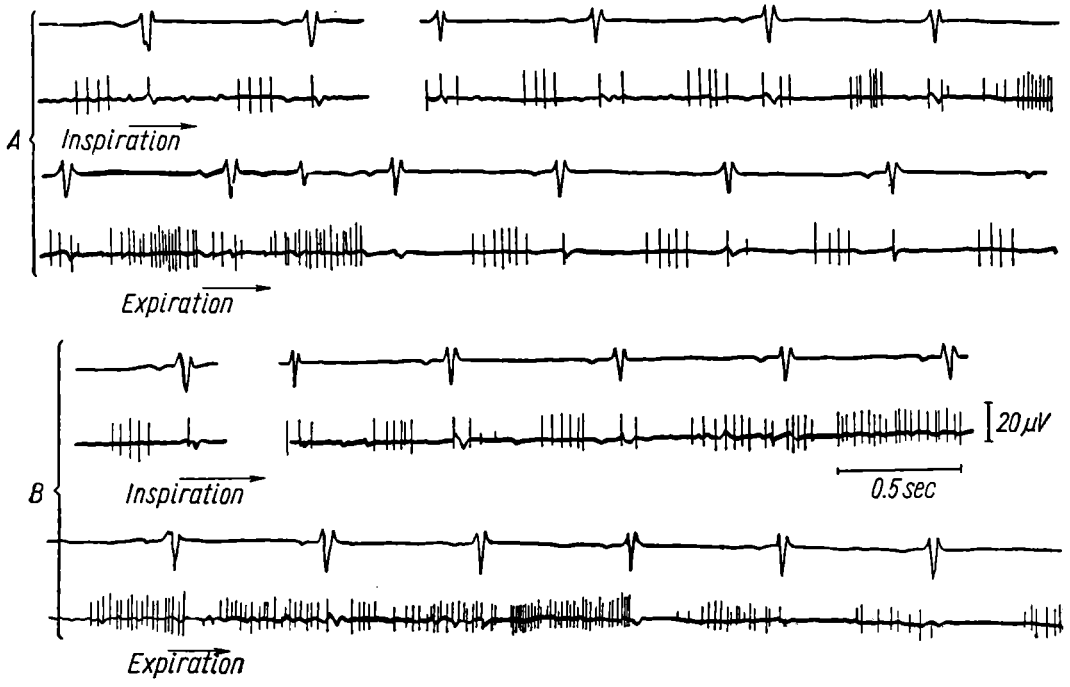

Fig. 4. Afferent impulse emission in three fibres leading from the mechanoreceptors of the right atrium exposed from the right cervical vagus nerve.

A—normal receptor activity during change in venous blood influx in respiratory cycle; *B*—activity of same receptors during compression of right descending coronary artery

venous influx is 4 in the systolic volley and 19 in the diastolic one (impulses of all receptors active at this moment are summed up). During coronary ligation the following changes in growth of the venous influx (in the course of the respiratory cycle) may be observed.

1. Earlier activation of the 2nd and 3rd receptors than normally.
2. Each volley consists of a larger number of impulses than the corresponding normal volley (during the respiratory cycle). In the diastolic volley this growth is larger.
3. The regularity of growth and decrease of the number of impulses is disturbed.
4. The volley configuration is changed.

Thus, in coronary ligation the number of impulses in the summary volley from all active receptors is considerably increased, the growth of the maximum diastolic number of impulses (28 impulses in given case) being greater than the increase in the minimum number of impulses per volley (6). It is also essential that in coronary ligation the volleys are so prolonged that they merge, following in an almost uninterrupted succession. This bears resemblance to the electroneurogram of the

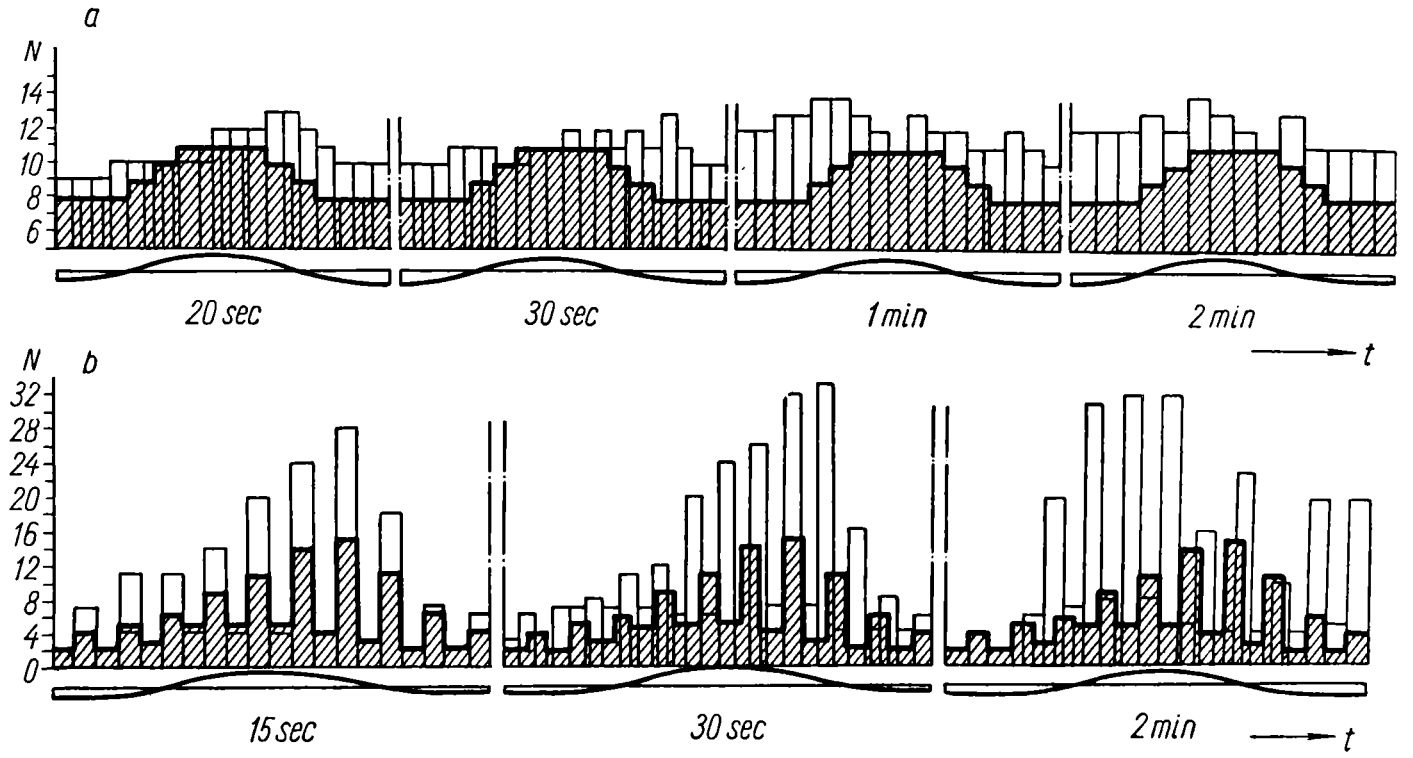

Fig. 5. Chart showing the relationship between the number of impulses (N) per volley during a change in the venous blood influx in the respiratory cycle: normal one is shown by shaded columns; that after compression of the right descending artery—by white columns.

Upper chart—activity of mechanoreceptor fibre whose impulse emission is shown in Fig.3; lower chart—activity of three fibres of mechanoreceptors whose impulse emission is presented in Fig 4. Undulated line—phases of respiration (downward curve expiration, upward curve—inspiration)

small atrial branch in which transition of volley activity to a continuous flux of impulses was discovered (see Fig. 1).

These observations indicate that the functions of the cardiac receptor apparatus just as the activity of other receptive formations are based on a quantitative principle determining the coding of information supplied to the central nervous system. The same quantitative principle underlies the functioning of the efferent system of the heart and vessels (M.G. Udelnov).

These findings may be interpreted as follows.

The atrial receptor responds to the mechanical deformation (tension) of the muscle fibre. The tension causes depolarization of the receptor, producing a generator potential (E. Granit) and, as a result of the latter, an impulse discharge. Consequently, an increase in the frequency of impulses during the emergence of an ischaemic focus may be due to a greater mechanical deformation (greater tension) of the muscle fibre and (or) the action of depolarizing agents, for example, ions of potassium, and acetylcholine, serotonine, which in myocardial infarction (as has been noted in clinical and experimental conditions) are secreted in quantity.

Tennant and Wiggers found that the ischaemic region during ligation of the coronary artery in dogs, far from ceasing to contract during a systole and even tends to extend. Bulging of the ischaemic focus was found in humans by means of roentgenokymography. M.G. Udelnov and E.A. Kyandzhuntseva showed also relaxation of the myocardium in the prenecrotic zone. An intact myocardium is to perform greater work since the ischaemic portion does not take part in the active systole. In addition, part of the work of the intact myocardium is spent on supporting the functionally defective regions. This results in a decreased stroke volume during an increased venous influx, dilation of the atria and incomplete drainage of the heart during a systole. In our experiments such an increase in the diastolic tension and an increase in the volume of the atria and ventricles during coronary occlusion were noted repeatedly. It seems probable that the above-described effect of increase in the number of impulses per volley results from the interaction of the following two factors: dilatation of the heart and the depolarizing action produced on the receptors by ions of potassium, acetylcholine, etc.

Whatever our interpretation of the findings made might be, however, it is clear that the changed character of the afferent signal is likely to cause reflex reactions which may account for a functional derangement in various systems. Chains of reflexes are brought into play to compensate for the deranged functions, whereas in case of a sufficiently strong stimulus and

inadequate compensatory mechanisms there is a risk of failure, i.e., decompensation. A change in the character of afferent impulses is the signal for these reflexes to come on the scene.

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THE ROLE OF UNCONDITIONED REFLEX MECHANISMS IN THE PATHOGENESIS OF MYOCARDIAL INFARCTION

B. S. KULAYEV

The clinical and pathophysiological data available at present are still insufficient for clarifying the cause of severe circulatory disturbances in the acute period of myocardial infarction. Moreover, the literature on this problem contains a lot of contradictory evidence as to the role played in the development of these disturbances by affection of the heart itself and by changes in the vascular bed. Thus, discussing the causes of the arterial pressure fall seen frequently in myocardial infarction some authors attribute the leading role in this process to cardiac insufficiency, others to the dilatation of the peripheral vessels. Compromise points of view exist also. Worthy of note are the insistent assertions by a number of competent authors (A.V. Vinogradov, 1962, et al.) that there is an increase in the summary peripheral resistance of the vascular bed in the acute period of myocardial infarction.

At the same time it is known that autopsy in fatal cases of myocardial infarction usually reveals vasodilatation in the visceral organs. The evidence of various authors concerning the reaction of the heart itself to myocardial ischaemia is contradictory as well.

In an attempt to settle these contradictions in experiments on animals, Bezold, A.V. Vinogradov and L.I. Dmitriyeva, A.V. Dokukin and others studied changes in the heart's action, the blood pressure and degree of the peripheral resistance, etc., under the influence of coronary occlusion. These efforts, however, proved unsuccessful, whereas the amount of contradictory evi-

dence grew. Some authors observed a reduction in the arterial pressure, while others encountered cases of its increase, in addition to tachycardia observed bradycardia, etc. At the same time, all these reactions to occlusion were, as a rule, brief, and the functions under study returned to their initial condition rapidly and for a long time.

These findings appear utterly inconsistent with our concepts of the heart as the leading reflexogenic zone responsible for the maintenance and unconditioned reflex self-regulation of the blood circulation. The coronary occlusion cannot be of no consequence for the numerous cardiac receptors. In its turn, a change in the afferent impulse emission from the heart cannot but have an effect on the state of the circulation.

In this connection the following questions had to be answered. Why in acute experiments on animals does coronary occlusion cause the effects so insignificant at first glance? Is its influence restricted only to these reactions in the arterial pressure? What is the explanation for the contradictory evidence on these problems in the literature? This is the subject of the present report based as it is on the results of experiments in which the effects of coronary ligation were compared with those of stimulating the ventricular receptors before and at different periods after ligation.

A total of 40 acute experiments on cats and 35 on rabbits were performed. Part of the cats were anaesthetized with urethane (0.7-0.1 g per kg of body weight), while the remaining cats under rauschnarcosis were subjected to low decerebration (method of immobilization had no substantial influence on the results of experiments). The experiments on rabbits were performed without general anaesthesia. The arterial pressure was registered in the carotid artery by means of a mercury and a membrane manometer simultaneously, respiration—by means of Marey's capsule connected to a tracheotomy cannula.

For stimulating the heart, the latter was by means of a special operation (Drinker, B.S. Kulayev) taken out through an artificial defect in the thorax, after which the edges of the exposed pericardium were sutured to the margins of the defect in the thorax, thereby restoring the hermetic condition of the pleural cavities and regaining the natural respiration. The cardiac receptors were stimulated with chemicals applied to the epicardium, usually with 0.4-1 ml of nicotine in a dilution of $1 \cdot 10^{-6}$ (weak stimulator) and $1 \cdot 10^{-3}$ (strong stimulator). For ligation of the coronary vessel (usually the left descending artery), it was exposed over a length of 0.5 cm, so as to introduce a ligature under it and occlude it at the required moment.

The findings made in these experiments are essentially as follows. Coronary ligation causes, as a rule, changes in the arterial pressure and the rate of the heart-beats and respiration, but the intensity and duration of these changes, however, are always less than the analogous reactions caused not only by a strong

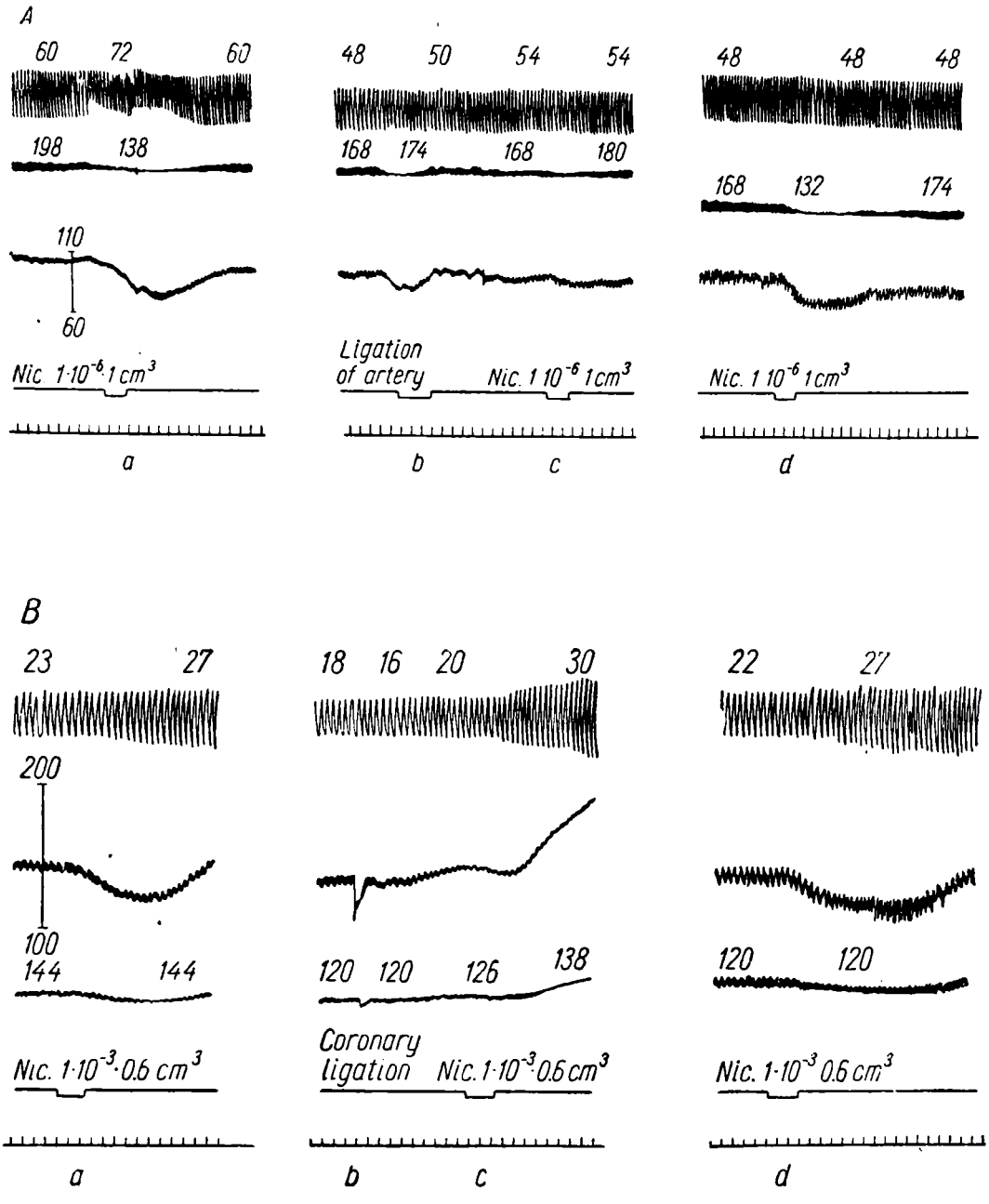

Fig. 1. Change in the reflex reactions of the arterial pressure to nicotine stimulation of the epicardial receptors before (*a*), immediately after (*c*), and 40 minutes after (*d*) coronary ligation (*b*).

A—in a cat in which stimulation of the epicardial receptors evoked only depressor reactions; *B*—in a cat in which slight stimulations evoked pressor, and strong ones, depressor reactions. The curves presented (from below downwards) are as follows: respiration, arterial pressure (mercury manometer), *AP* (membrane manometer), stimulation mark, time mark (5 sec). The figures above the respiration and membrane manometer curves indicate the rate of respiration and heart beats per sec respectively. The inscriptions above the stimulation mark indicate concentration of nicotine used for stimulation of the epicardial receptors and other interventions. The vertical line—*AP* scale

but also by a moderate and sometimes even by a weak chemical stimulation of the epicardial receptors (Figs. 1, 2, 3). Within a few seconds of ligating the coronary artery, the arterial pressure returns to the initial and even to a higher level at which it may remain for a long time. The greatest changes within the first hour of ligating the coronary artery occur in the reflexes evoked by stimulation of the cardiac receptors. These reflexes prove to be markedly inhibited immediately after ligation of the arteries: weak and moderate stimulations evoke no reflexes at all, the reflexes to strong stimulations are markedly weakened, and sometimes distorted. In 20-40 minutes' time after coronary ligation the character and intensity of the reflexes are restored to a certain extent.

For a clear understanding of this result a more detailed analysis of the experimental material is required. The first step should be to find out what determines the character of the arterial pressure reaction to coronary ligation.

In rabbits, in which stimulation of the cardiac receptors always evokes depressor reactions (V. N. Chernigovsky, B. S. Kulayev) coronary ligation also causes a decrease in the arterial pressure, which is more evident in the animals in which the chemical stimulation of the epicardial receptors before ligation produced strong vasomotor effects.

The same effect was discovered in cats, both in those in which chemical stimulations of the cardiac receptors of variable intensity had caused only depressor reactions (Fig. 1*A*) and those in which weak stimulations had caused pressor and strong stimulations—depressor reactions (Fig. 1*B*). In the latter case, however, biphasic reactions might occur in response to coronary ligation: a reduction and then an increase in the blood pressure. In those cats in which stimulations of the cardiac receptors of variable intensity evoked only pressor reactions, coronary ligation evoked a pressor reaction (Fig. 2) (initial sharp reduction of the arterial pressure was due to the heart being pulled up at the moment of coronary ligation).

Thus, the depressor or pressor character of response to ligation is determined by the same factors which are responsible for the type of reflexes to chemical stimulation of the epicardial receptors. In our previous papers we adduced a number of arguments to prove that the principal role among these factors is played by the state of the vasomotor centre, which, in its turn, is determined by the amount of afferent impulses conveyed to it (B.S. Kulayev, 1954, 1958). This is proof that at least the vasomotor component of the reaction has a reflex origin, and is effected through the centres of the medulla oblongata. At the same time, it was found that a change in the arterial pressure in response

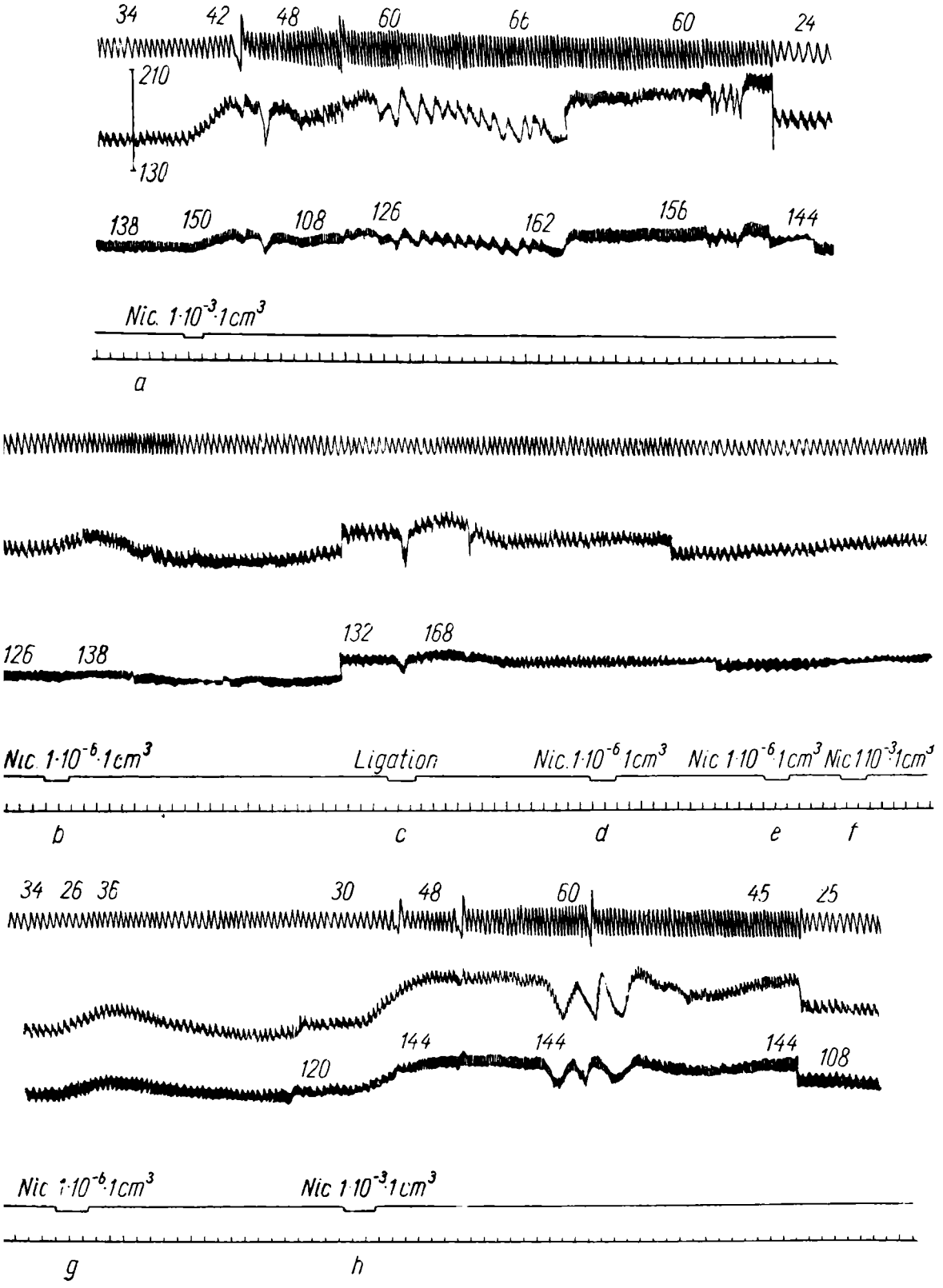

Fig. 2. Change in the reflex reactions of *AP* during stimulation of the epicardial receptors with nicotine in different concentrations before (*a*, *b*), immediately after (*d-f*) and 45 min after (*g-h*) coronary ligation (*c*). Same designations as in Fig 1

to coronary ligation was identical in character with the reaction to a strong chemical stimulation of the epicardial receptors whereas in intensity it proved very weak. It should be added that the relation between the degree of this reaction and the level at which the coronary artery was ligated, i.e., the size of the ischaemic focus proved inverse in the majority of cases: the larger the ischaemic focus, the less the reflex reaction to the arterial pressure.

The discovered dissociation between the character and intensity of the reactions under discussion is directly connected with those reflex changes following in their wake which are brought into play by stimulation of the cardiac receptors. In those experiments in which prior to coronary ligation only synonymous reactions were noted, irrespective of the intensity of chemical stimulation of the epicardial receptors, ligation was usually followed by cessation of reaction to weak stimulations, while in the case of strong stimulation the reactions sharply decreased and became similar to those seen during weak stimulation (see Fig. 2).

In other experiments in which prior to coronary ligation weak concentrations of nicotine had produced a pressor and strong ones a depressor effect, reactions to weak chemical stimulation also disappeared and those to strong stimulation were often distorted, transforming from depressor into pressor reactions (Fig. 1*B*; Fig. 3). In those cases where experimental infarction developed slowly it was possible to follow the details of this transformation (see Fig. 3). Within a few seconds of ligating the coronary artery the depressor reaction to stimulation of the epicardial receptors with nicotine at a concentration of $1 \cdot 10^{-3}$ (strong stimulation) weakened and in case of repeated stimulations transformed in 8 minutes' time into a pressor reaction resembling that which prior to ligation (Fig. 3*B*) had been evoked by a weaker stimulation ($1 \cdot 10^{-4}$); in 12 minutes' time the vascular component of the reaction disappeared altogether, while the heart's reaction was one of tachy- rather than bradycardia. In 50 minutes, the initial character of the reaction was restored although it remained weaker somewhat.

There is usually an inverse ratio between the intensity of the reaction of the arterial pressure to coronary ligation and the degree of inhibition of epicardial reflexes caused by this interference: the greater the reaction, the less the inhibition, and vice versa.

The experimental material presented here (on cats and rabbits) contains these two major facts: (1) the arterial pressure reaction to coronary ligation is identical in character with the reflex reaction to a strong stimulation of the epicardial recep-

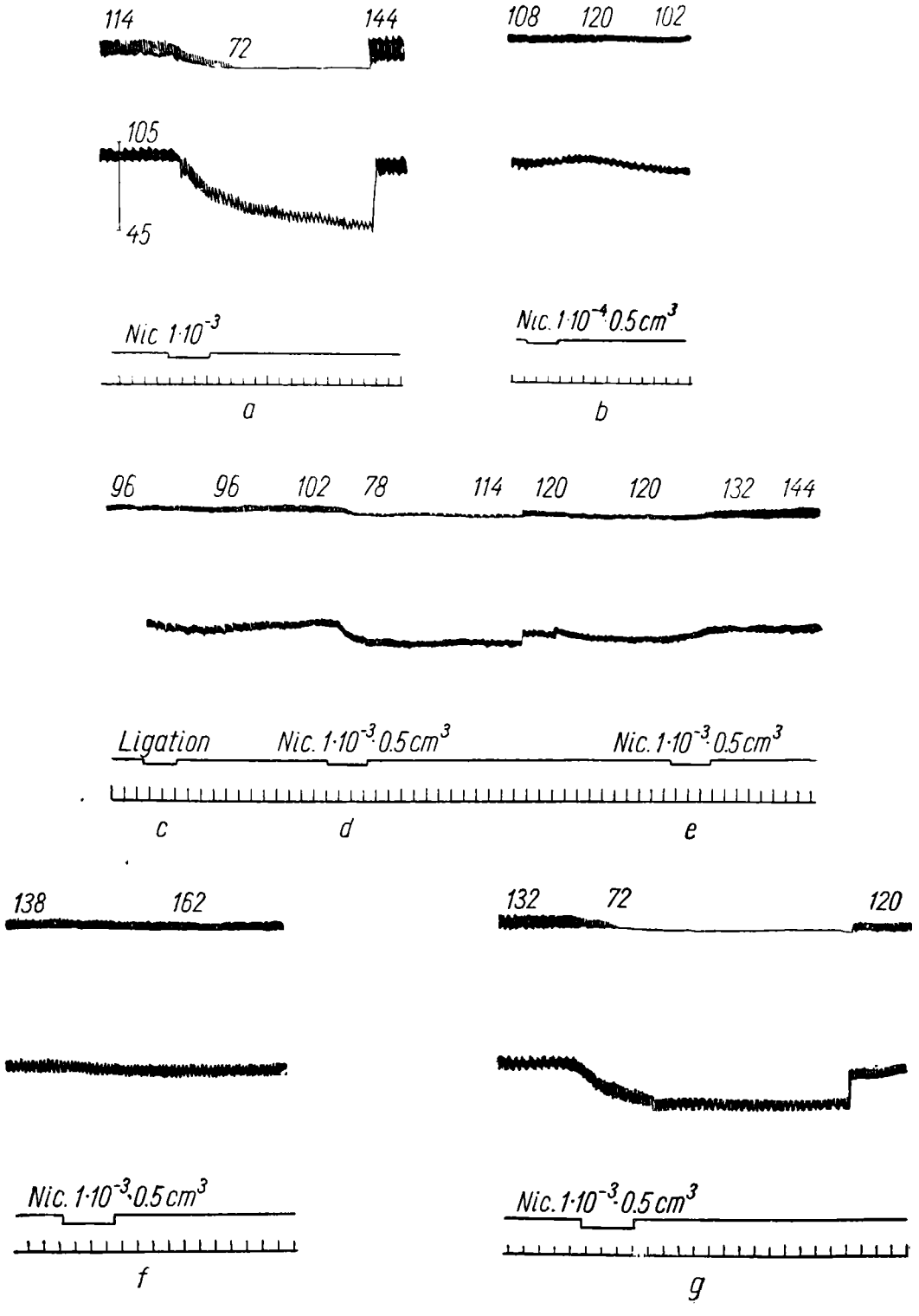

Fig. 3. Change in the reflex reactions of *AP* to stimulation of the epicardial receptors before (*a*, *b*), immediately after (*d*), 8 min (*e*), 12 min (*f*) and 50 min (*g*) after coronary ligation (*c*). From above downwards: *AP* (membrane manometer), *AP* (mercury manometer), the rest is the same as in Figs. 1-2

tors, but is sharply different from the latter in its less intensity and shorter duration; (2) following coronary ligation the reflexes from the cardiac receptors to the arterial pressure markedly abate or disappear altogether. There is full reason to presume that both these facts are due to one and the same cause—a disturbance in the blood supply to the heart.

Inhibition or disappearance of reflexes from the heart to the arterial pressure may result from affection of one of the three principal links in the reflex arc. We have no grounds to suppose that any changes took place in the efferent link. Changes in the centres are also unlikely under the given experimental conditions. If as a result of coronary ligation any impulses appearing in the cardiac receptors had been capable of inhibiting the vasomotor centre, we should have observed the growth of depressor reactions, and conversion of pressor reactions into depressor ones (B.S. Kulayev, 1954, 1959; I.M. Rodionov, 1957). However, in the case of weakened reactions, we observed the opposite phenomenon: either inhibition of the depressor reactions and their conversion into pressor ones or complete extinction of the reflex. It means that the cause of disappearance of the reflexes should be sought in their afferent link, i.e., in the cardiac receptors.

The meagre information we have on changes in the afferent impulses from the heart as a result of coronary occlusion is contradictory. Stuppler failed to discover substantial changes in this case. Kaindl and his co-authors have reported an intensification, E.G. Petrova an abatement, F.P. Yasinovskaya both intensification and inhibition of the afferent impulses from the heart to the degree of their complete extinction. It follows from analysis of these literary data that the afferent impulses either remained unchanged or became intensified after coronary occlusion, more often in those cases when they were registered in the nerves connected mainly with the atrial receptors. When the afferent impulses were recorded from the nerves connected with the ventricular receptors (E.G. Petrova, F.P. Yasinovskaya), the authors observed inhibition or cessation of these impulses. According to F.P. Yasinovskaya, this was usually preceded by a brief yet intensive increase of the afferent impulses. It is precisely in the nerves of the ventricles that the changes caused by myocardial infarction (casuistics: atrial infarction) should be sought.

The literary data cited here suggest the following interpretation of the results of our experiments. The outbreak of afferent impulses occurring immediately after coronary occlusion evokes that particular arterial pressure reaction which has been observed by us and other authors. The intensity of this volley

of afferent impulses from the heart is responsible for the identical character of the reflex reaction in response to ligation and the reaction to a strong chemical stimulation of the epicardial receptors. The brevity of the volley as well as the subsequent prolonged inhibition or disappearance of impulses in the region affected by the infarction determine, on the one hand, the weakness and brevity of the arterial pressure reaction to coronary occlusion and, on the other hand, account for the disappearance of reflexes from the ventricular receptors of the affected heart. The larger the calibre of the ligated coronary artery, the more probable is the rapid transition of the initial increase in impulse intensity to complete impulse cessation. Therefore, the existence of a proportional relation between the diameter of the ligated artery and the intensity of the arterial pressure reaction is not obligatory, and even an inverse ratio may be noted, as has often been the case in our experiments. At the same time, the higher the site of ligation of the artery (the larger its diameter), the wider the infarction area, and consequently the more ventricular receptors lose their excitability, and the greater the degree of inhibition of the afferent impulses from the heart, the more complete is the disappearance of the vascular reflexes to stimulation of the cardiac receptors.

Some authors describe intensification of the afferent impulses from the atria following coronary occlusion. This is apparently a secondary phenomenon occurring as a result of extension of the atria with the progression of cardiac insufficiency. The increase of afferent impulses from the atrial receptors can partly compensate for the inhibition of the ventricular afferent impulses, but the degree of this possible compensation should be small because the ventricular impulses play the most important role in the maintenance of the vascular tone (B.S. Kulayev). In patients with cardiosclerosis or atherosclerosis such an increase of the afferent impulses from the atria may be absent entirely (I.E. Ganelina and B.S. Kulayev).

It follows from the above that the general haemodynamic changes in experimental myocardial infarction are to a considerable extent determined by disturbances in the receptor function of the affected heart. The degree of such disturbances should be judged not by the intensity of reaction to occlusion of the coronary vessel but by the degree of inhibition of the cardiac reflexes occurring under its influence.

Partial deafferentation of the heart in healthy animals leads to a stable increase in the arterial pressure (Waele, Velde; V.N. Chernigovsky; B.S. Kulayev; D.L. Dligach, Z.S. Dolgun, B.S. Kulayev). This reaction is based on an increase in the summary peripheral resistance associated with a considerable re-

distribution of the blood within the body (B.S. Kulayev). Occlusion of the coronary vessels in acute experiments on animals leads, according to evidence by A.V. Dokukin, to vasodilatation of certain visceral organs as well as to vasoconstriction of the extremities. In myocardial infarction, the increase in the peripheral resistance entails an arterial pressure increase only in the early period after onset, which is followed by a considerable decrease in the arterial pressure, often below the initial level, with the aggravation of cardiac insufficiency. Under these conditions, the increase in the summary peripheral resistance owing to partial deafferentation of the heart should play a positive role, preventing the development of a cardiogenic shock. More complete deafferentation of the heart, however, leads to normalization of the cardiac levels of the arterial pressure with constant oscillations and frequent significant declines below the initial level (D.L. Dligach, Z.S. Dolgun, B.S. Kulayev). This effect is probable under the conditions of an extensive myocardial infarction, particularly in cardiosclerosis when a considerable part of the receptors undergo degeneration. In such cases, the body remains defenseless against severe cardiac insufficiency, which is liable to lead to a cardiogenic shock complicated by a vascular tone insufficiency.

The disturbance in the vascular reflexes from the heart occurring in myocardial infarction affects the heart itself due to affection not only of the afferent but also of the efferent link in the reflex arc. V.S. Livshits showed that affection of the myocardium leads to a sharp reduction in its sensitivity to the arriving efferent impulses, which, according to M.G. Udelnov, provides conditions for distortion of the effect of extracardial influences, provoking tachycardia instead of bradycardia. Indeed, we have observed such cases following ligation of the coronary arteries (see Fig. 1*B*; Fig. 3). This sheds light on the mechanism of origination of tachycardia, an unwelcome phenomenon in myocardial infarction.

The concepts developed in this article concerning the mechanisms and the possible role of disturbances in the unconditioned reflex mechanisms maintaining the arterial pressure in the pathogenesis of myocardial infarction require further experimental and clinical verification. The findings already available, however, and the coincidence of some of our conclusions with the evidence furnished by certain clinicians (A.V. Vinogradov, et al.) warrant our hopes of success in the use of the suggested approach to the experimental solution of the problem.

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THE CLINICAL CHARACTERISTICS OF DISTURBANCES OF THE ANTICOAGULATIVE MECHANISMS IN ATHEROSCLEROSIS AND MYOCARDIAL INFARCTION

E. I. CHAZOV

It will be no exaggeration to say that one of the most important problems of modern medicine both from the theoretical and practical points of view is the problem of thromboses in general and the problem of coronary thrombosis in particular. The urgency of this problem is accounted for not only by the high incidence of thrombosis which is responsible for example for 80 per cent of lethal cases of atherosclerosis and hypertension (V.S. Smolensky), but also to insufficient information on the mechanism of this process. The inadequacy of our knowledge in the field of pathogenesis of thromboses is partly responsible for the fact that until recently we have not known effective methods for their therapy. Thrombosis usually develops with cardiovascular diseases, in the overwhelming majority of cases during atherosclerosis. This is particularly true for coronary thrombosis, as is evidenced by numerous statistical data (N.N. Anichkov et al., A.L. Myasnikov, A.I. Strukov et al., I.V. Davydovsky, P.E. Lukomsky and E.M. Tareyev, Wright et al., etc.). The existing theories ascribed thrombus formation to those changes which dominate in the pathological process. For example, in cardiac insufficiency the origination of thrombosis was attributed to a slowing of the blood current.

In atherosclerosis, however, the principal role was attached to the development and disintegration of plaques which are responsible, in the opinion of advocates of this theory, for the appearance of rough surfaces on which settle and disintegrate the formed elements giving rise to intravascular thrombus formation (N.N. Anichkov et al.).

A well-known authority on thrombus formation, Quick, believes that the major factor preventing blood clotting is the ideally smooth, unmoistened surface of the vascular endothelium.

At the same time, the day-to-day practice of clinicians and pathoanatomists caused doubts concerning the decisive role of morphological vascular changes in the development of thrombosis. Suffice it to point out that almost every physician and pathoanatomist observed severe, far-advanced cases of atherosclerosis with considerable morphological changes—ulceration of plaques, development of calcinosis in the coronary vessels in which, at the same time, no thrombi were discovered. On the other hand, fatal cases of thrombosis are known to have occurred at a young age with no significant changes in the vessels. Postoperative thrombus formation can also be hardly explained from the "morphological" viewpoint.

That is why so much hope has been aroused by the attempts to connect pathological thrombus formation with an increase in the blood-clotting factors. Over the past twenty years, our concepts of the blood-clotting system have been made wider and more accurate, new discoveries have been made, and each new finding seemed to bring us closer to exposing the causes of thrombus formation. It suffices to recall the extensive literature in which some authors attributed thrombus formation to an increase in the prothrombin content of the blood and even regarded the latter as a sign of the "preinfarction" state. Later, however, these views proved groundless. Indeed, how can one attribute thrombus formation to an increase in the prothrombin content of the blood when there is enough clinical evidence to indicate the possibility of thrombus formation during anticoagulant therapy at low prothrombin indices (B. V. Konyayev)? Pollard and his associates observed thrombus formation in nine cases even in the presence of haemorrhagic diathesis caused by anticoagulant therapy.

On the other hand, a very significant (up to 180 per cent) increase in the prothrombin content of the blood, which was induced in healthy animals by B. A. Kudryashov and his collaborators, was not accompanied by clot formation. According to the theory attributing thrombus formation to an increase in the level of the blood-clotting agents, intravenous injection of the latter should immediately result in intravascular thrombus formation. Many reports are known, however, about injections of thrombin (known to be the final enzyme in the blood-clotting chain) to healthy animals which were not followed by the development of thrombosis (Gerendas a. Csapo, Jürgens a. Studer, Vandenbroucke a. Verwilghen).

Embarking in 1958, at the suggestion of A. L. Myasnikov, on

Fig. 1. Microphotograph. A thrombus in the coronary vessel of a healthy rabbit after administration of thrombin and pituitrin. Magnification 150 times

the study of the pathogenesis of thromboses and development of new methods of therapy, we realized the need to search for new ways in the solution of these problems. The theoretical basis for such a search was the theory of the physiological anticoagulative system of the body advanced in those years by Prof. B. A. Kudryashov and his collaborators. They showed in a series of experiments that biochemical changes in the blood raising the predisposition to thrombus formation cause a protective anticoagulative reaction manifested by a considerable increase of the blood heparin and fibrinolytic activity. In case of insignificant changes in the blood-clotting factors the compensation is effected at the expense of the anticoagulative and lysis-producing agents circulating in the blood; in case of the danger of thrombus formation an anticoagulative reaction of a neurohumoral character is brought into play.

During our research we set about the clinical and experimental study of the state of the anticoagulative mechanisms in those

pathological processes which are most frequently accompanied by thromboses.

A number of authors (I.V. Davydovsky, G.F. Lang, A.V. Smolyannikov, and others) attribute great importance in the development of coronary thrombosis to functional disorders, a vascular spasm. S.S. Weil, for example, succeeded in producing hyaline thrombi in small vessels of the heart by stimulating the cervical sympathetic ganglia. D.D. Pletnev writes in his monograph: "The question comes to mind involuntarily, whether thrombosis is the cause of a pain attack or it develops during the latter as a result of a prolonged spasm of the sclerotically changed coronary vessels. I for one am in favour of the latter supposition."

That is why we were interested above all in the state of the anticoagulative mechanisms during a vascular spasm. A healthy animal simultaneously given thrombin and also pituitrin which is known to cause a coronary spasm will develop an acute coronary insufficiency due to the formation of a clot in the coronary vessel (Fig. 1). The further fate of the thrombus, however, depends on the degree of drug influence. In the case of large thrombin and pituitrin doses (4 ml of thrombin solution with 12 sec activity and 0.5 ml of pituitrin solution) rabbits, as a rule, died within the first two hours, dissection usually revealing fresh thrombi in small coronary arteries in 40-50 per cent of cases. It is interesting to note that in these experiments an anticoagulative reaction took place which was manifested by a sharp increase in the heparin and fibrinolysin content. In such cases, the blood lost its capacity to clot altogether (Fig. 2).

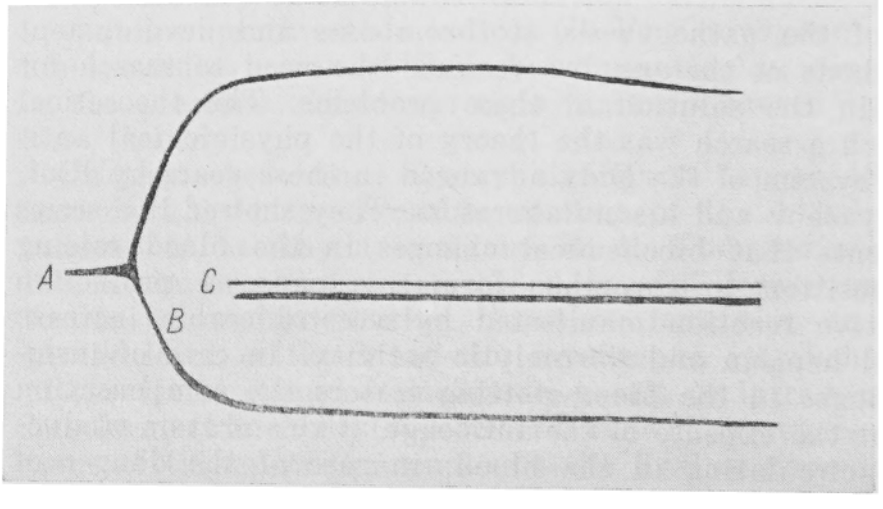

Fig. 2. Thromboelastogram taken after induction of a thrombus in a normal rabbit.

A—before thrombin injection; *B*, *C*—after thrombin injection

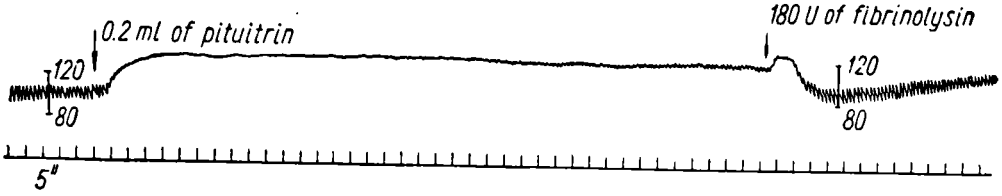

Fig. 3. Spasmolytic effect of fibrinolysin in a study of the coronary circulation by the method of humoral isolation

To draw a comparison between experimental and clinical findings, L.F. Nikolayeva of the Institute of Therapy under the USSR Academy of Medical Sciences studied the free heparin content of the blood in patients with angina pectoris and those with pains of extracoronary origin. It was found that in patients of the second group the heparin content remained unchanged whereas patients of the first group showed its considerable increase above the control figures. Thus, the appearance of a vascular spasm contributing to thrombus formation was accompanied by a defensive reaction manifested by an increase in the heparin content of the blood. It is known that heparin dilates the coronary vessels. As has been found in the investigations which A. V. Trubetskoi and we have carried out recently, the same quality is possessed by fibrinolysin which eliminates the coronary spasm caused by pituitrin (Fig. 3).

It is probable that the anticoagulative reaction exerts a favourable influence on the coronary circulation not only by destruction of the fresh fibrin filaments, i.e., a fresh thrombus, but also through its possible spasmolytic effect. The possibility of coronary thrombosis in healthy animals in connection with a pituitrin spasm was corroborated after the publication of our findings by other Soviet and foreign authors (V.V. Vasilyeva, K.M. Lakin; de Martini). The experiments carried out indicated the possibility of a natural destruction of the newly-formed thrombi by the action of the anticoagulative system of the body. We had good reasons for such an assumption. It was found that following an injection of smaller doses of thrombin (2 ml of solution with 12 sec activity and 0.3 ml of pituitrin solution) ECG changes pointing to the onset of an acute coronary failure disappeared within the first hour. The observations carried out jointly with Y.G. Tinyakov showed that dissection of animals within a few hours of injecting thrombin with pituitrin revealed fresh thrombi in the coronary vessels approximately in 50 per cent of cases, whereas the coronary vessels in animals killed after 24 hours proved patent almost in all cases.

To verify the fact of thrombus destruction in the coronary

Fig. 4. Dissolution of a thrombus in the coronary vessel of a healthy dog 4 hours after its induction

vessel we undertook a second series of observations in which thrombus formation was provoked by a direct injection of heated thrombin into the coronary artery of the dog. In these cases also, we failed to discover thrombi in the heart vessels of animals killed after 24 hours. During an earlier observation, in 4-5 hours' time after inducing a thrombus, one could even observe its destruction (Fig. 4). The possibility of spontaneous destruction of fresh thrombi in healthy animals is confirmed by the observations of foreign authors, for example, Kwann and his collaborators. It is probable that a similar phenomenon may take place in a healthy human being, and in general when the anticoagulative system of the body has an adequate functional capacity.

It is known that coronary thrombosis develops, as a rule, in atherosclerosis. We were, naturally, interested in the state of the anticoagulative mechanisms during this disease and the possible association between their disturbances and the development of thrombosis. As has been shown by experiments, after

inducing cholesterol atherosclerosis in rabbits after N.N. Anichkov's method the progression of the pathological process is accompanied by a decrease not only in the level of free heparin and fibrinolytic activity of the blood but also in the capacity for their compensatory increase during biochemical changes in the blood increasing the predisposition to thrombus formation. Similar findings were made also in Prof. B.A. Kudryashov's laboratory during experiments on rats kept on an atherogenous diet likely to cause coronary thrombosis. Reports of inhibition of fibrinolysis in rabbits with experimental atherosclerosis may also be found in the foreign literature (Srivastava a. Charavarti). We as clinicians were interested above all in the state of the humoral agents of the anticoagulative system, in particular, heparin and fibrinolytic activity in atherosclerotic patients. In collaboration with L.F. Nikolayeva and T.L. Kozhevnikova, we investigated these factors in more than 200 patients with atherosclerosis of a variable dergee. At the same time, we managed to note a decrease in the activity of the above-mentioned anticoagulative factors in relation to the degree of atherosclerotic changes (Fig. 5). In the lst stage of atherosclerosis with incipient signs we failed to discover marked changes in the heparin content and fibrinolytic activity. In 22 out of 100 cases one could note a decrease in the heparin content of the blood, and in 30 cases a decrease in the fibrinolytic activity. At the same time, in 27 cases, the heparin content was increased, while the fibrinolytic activity of the blood was heightened in 21 cases. It is probable that an important role in these differences is played by the individual characteristics of the body, its compensatory potential. It is doubtless that the group of patients with atherosclerosis in the lst stage widely differ from one another with regard to their functional and biochemical characteristics. Indeed, this group includes both patients with incipient signs of the disease who had only begun to suffer from attacks of angina pectoris shortly before their admission to hospital and patients with a relatively severe, protracted course of the disease, but with no resultant changes in the myocardium.

It should be presumed that an increase in the heparin content of the blood and the level of fibrinolytic activity in a number of patients with atherosclerosis of the lst degree has a protective character. In such cases, the compensatory potential is still great enough and gives rise to a corresponding reaction to a particular complex of functional and biochemical changes responsible both for a vascular spasm and changes in the blood-clotting system. This group of patients, however, may already show changes pointing to inhibition of the anticoagulative mechanisms.

These changes are considerably larger and oftener in the 3rd

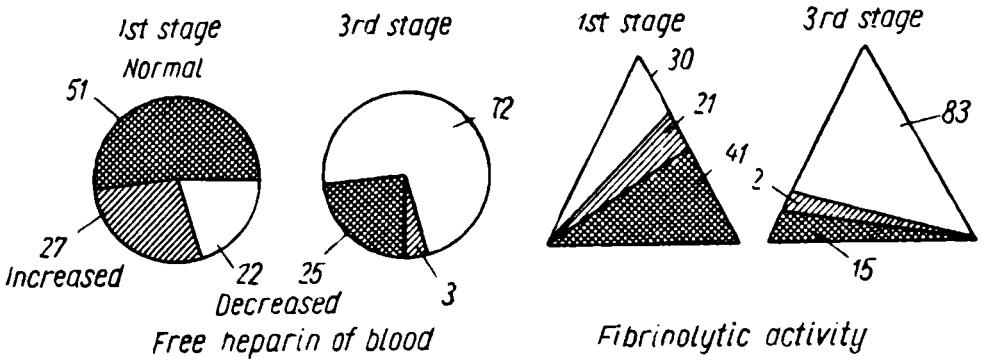

Fig. 5. Diagrams of changes in the heparin content of the blood and fibrinolytic activity in patients with atherosclerosis (figures indicate number of patients)

stage of the disease. This group included patients with marked atherosclerotic changes who, as a rule, had sustained myocardial infarction in the past and been admitted to the Institute with severe attacks of angina pectoris. Among these patients we observed a decrease in the free heparin content of the blood in 72 cases out of 100, and a reduction of the level of fibrinolytic activity in 83.

What is the cause of these changes? It is known that both the level of heparin and heparinoids and the fibrinolytic activity of the blood depend on the state of the vessel wall, the activity of the reticuloendothelium. It is doubtless that with the progression of atherosclerosis this function of the vessel wall will be impaired. For example, a number of authors have noted hypoplasia and decrease in the number of heparinocytes during atherosclerosis (L.P. Yermilov, Fernex, Constantinidis a. Cairns, etc.). Some reports contain evidence pointing to a change of the fibrinolytic activity in the case of pathological changes in the vessel (Astrup, Perlick, Albrechtsten). The regulation of the level of fibrinolytic activity and heparin content of the blood is effected also by specific inhibitors contained in the blood of animals and man—antiheparin and antifibrinolysin. As has been shown by our observations on 30 patients with the 1st stage of atherosclerosis and 30 patients with the 3rd stage the degree of inhibitory influences with regard to heparin and fibrinolysin grows with aggravation of atherosclerotic changes. Changed but little in the 1st stage of the disease this inhibition considerably increases in the 3rd stage of atherosclerosis (Fig. 6).

It is doubtless that an increase in the antiheparin and antifibrinolytic activity of the blood is one of the factors responsible for the high incidence of thrombosis in atherosclerosis.

Thus, the origination of thrombosis in coronary atherosclerosis

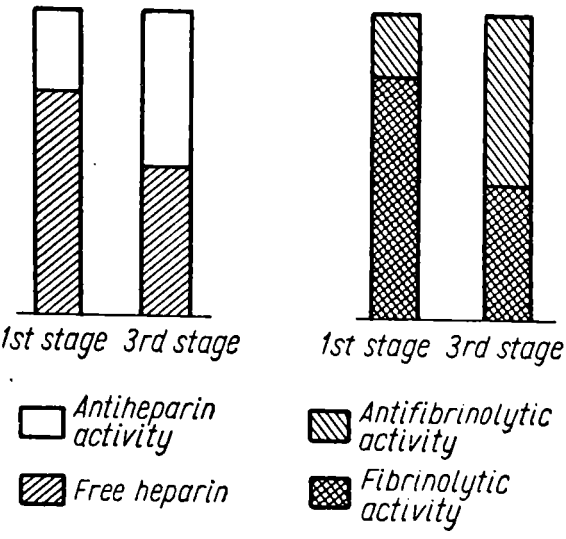

Fig. 6. Antiheparin and antifibrinolytic activity of the blood in patients with atherosclerosis

is a complicated process which cannot be ascribed to morphological changes alone. An important role in the high incidence of thrombosis in atherosclerosis, in addition to the atherosclerotic vascular changes in their morphological aspect, is undoubtedly played by the functional and biochemical disturbances, in particular by inhibition of the anticoagulative mechanisms as a manifestation of depression of the anticoagulative system of the body.

Such views increasingly gain ground in clinical studies, whose authors confirm the fact of disturbance of the anticoagulative mechanisms in atherosclerosis and attribute the high incidence of thrombosis to these changes (L. P. Yermilov, T. L. Kozhevnikova, L. F. Nikolayeva, V. M. Panchenko, A. Y. Yaroshevsky, and E. K. Zhavoronkova, Astrup, Hume, Szczeklik et al., etc.).

Of great interest is the study of the state of the anticoagulative mechanisms in the acute period of myocardial infarction, which makes it possible to uncover the mechanism of origination of coronary thrombosis. The heparin content of the blood, and the level of fibrinolytic activity were studied in 100 patients with myocardial infarction. In half of them a thromboelastographic investigation of the blood was carried out.

During the study of the anticoagulative mechanisms in this group of patients one could note at first glance a large variety of symptoms. Their comparison with the clinical picture, however, revealed a distinct relationship between them and the specific features of the pathological process. First of all, in 68 patients we discovered an increased free heparin content of the blood in the acute period of myocardial infarction. This increase varied from a low level to that which was $1^1/_2$-2 times the normal. It was higher in young persons with myocardial infarction and when the attack of pain was at its height. The pain was, as a rule,

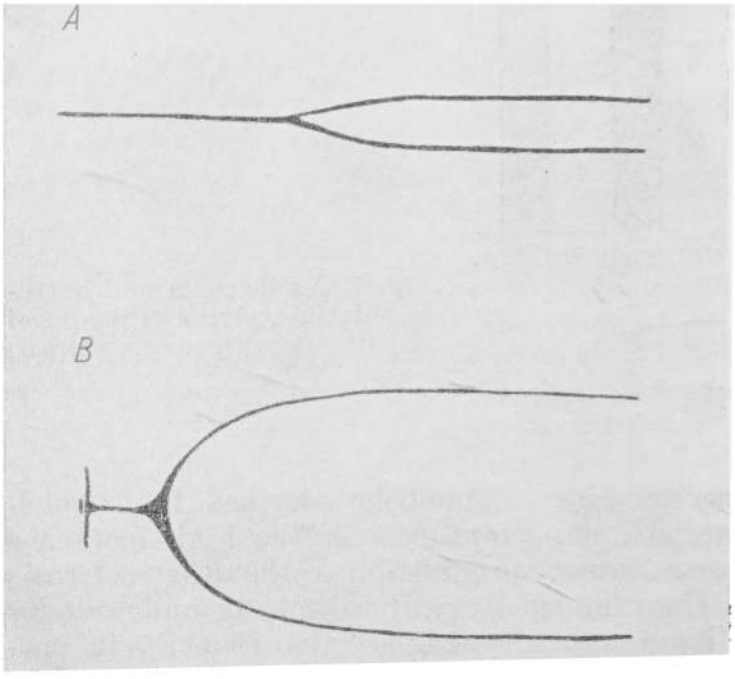

Fig. 7. Thromboelastogram of a patient with myocardial infarction.
A—during attack of pain; *B*—after attack of pain

accompanied also by an intensified fibrinolytic activity of the blood. Of the 25 patients admitted to the Institute with a severe status anginosus 23 showed a considerable increase in the fibrinolysin concentration to the degree of complete lysis of the clot in thromboelastography. In ten cases, the fibrinolysin content was raised during the first hours and in the usual course of myocardial infarction. Such increase, however, was later followed by inhibition of the fibrinolytic activity of the blood (Fig. 7).

It is interesting to compare these findings with the observations of certain pathoanatomists on the incidence of thrombosis in relation to the life-span of patients with myocardial infarction. For example, Spain and Bradess discovered thrombi in the coronary arteries of persons who had died within the first hour of acute coronary insufficiency only in 16 per cent of the cases, whereas in 24 hours' time this proportion grew to 54 per cent. Approximately the same figures are cited by A. M. Vikhert and his collaborators. It becomes possible to associate to a certain extent the lower incidence of thrombosis in persons who died within the early hours of the onset of myocardial infarction with the function of the anticoagulative system. The fact that the development of myocardial infarction brings into play a

protective anticoagulative reaction is also indicated by the increase in the heparin content of the blood in the acute period of infarction. Mention of such an increase has been made in the recent studies of a number of authors (A. A. Bagdasarov et al., L. F. Nikolayeva, L. P. Yermilov). L. P. Yermilov noted during autopsy of such patients an original reaction on the part of the heparinocytes indicating an increase in their function and their stimulation. The same protective reaction on the part of the heparinocytes has been observed in thrombosis by Pepler and Meyer.

One naturally begins to wonder why in spite of the reaction on the part of the blood heparin thrombi nevertheless do form in the coronary vessels. We believe that in the case of coronary thrombosis this reaction is obviously inadequate for preventing intravascular thrombus formation although it may possibly limit the progression of thrombosis.

In the acute period of myocardial infarction, 67 patients were found to have a considerable reduction of the fibrinolytic activity of the blood noted since the first hours of their admission to the Institute. And even those 33 patients who had been admitted with high indices of fibrinolytic activity showed rapid

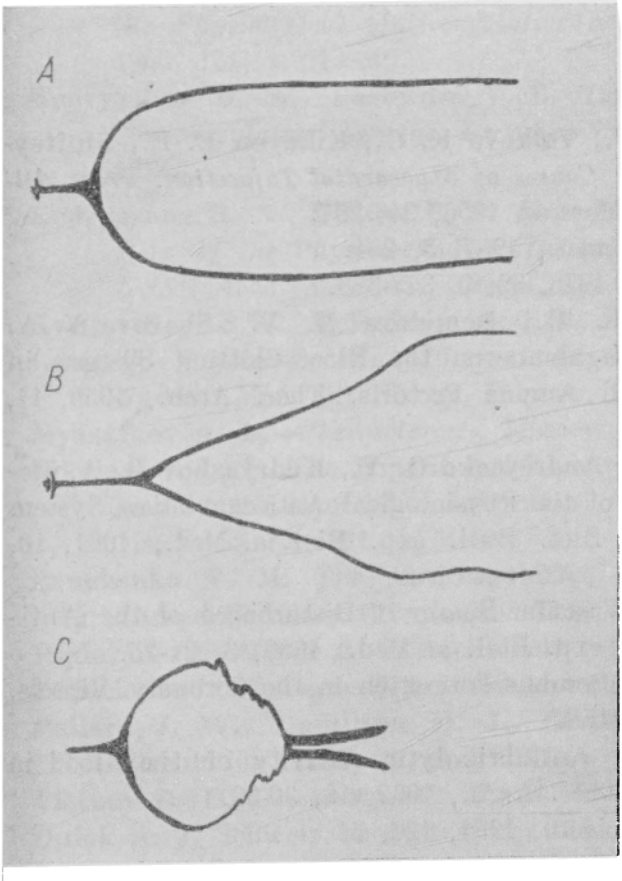

Fig. 8. Thromboelastogram of patients in the early hours of myocardial infarction.

A—usual course; *B*—during collapse; *C*—during severe status anginosus

changes toward reduction of the fibrinolytic activity. These disturbances, corroborated by the findings of Hume, Astrup, Szczeklik, T.L. Kozhevnikova, and V.M. Panchenko, play, in our opinion, an important role in the process of thrombus formation within the heart vessels (Fig. 8).

We have noted an especially significant inhibition of the anticoagulative mechanisms in patients with collapse during myocardial infarction. All of 28 such patients were found to have low indices of the blood heparin and fibrinolytic activity, thrombi in the coronary vessels being found at autopsy in 10 out of 13 fatal cases, whereas in another six fatal cases in the group of patients without collapse thrombi were discovered only in one.

It can be inferred from the study of the pathogenetic mechanisms of thrombosis that in the process of thrombus formation in the coronary vessel an important, if not decisive, role is played by a disturbance in the protective anticoagulative mechanisms, namely, their inhibition. These findings serve to outline some rational ways of therapy of thrombosis by intensifying the anticoagulative reactions through an increase in the heparin content of the blood and the level of fibrinolytic activity, which is particularly important for patients with a cardiovascular collapse.

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STUDY OF THE FIBRINOLYTIC ACTION OF UROKINASE

G. V. ANDREYENKO, E. I. CHAZOV

The advances made of late in the study of the blood-clotting mechanism, the discovery and investigation of the reflex-humoral physiological anticoagulative system have made it possible to use information obtained in this field of research in the diagnosis and treatment of a number of diseases. For example, the use of direct- and indirect action anticoagulants has been an important step toward the control of thromboembolic diseases. This, however, could not provide a solution to the problem of treatment and prevention of thromboses, since the administration of anticoagulants can do no more than limit the extension of the morbid process, partly interfere with the formation of new thrombi (by use of large doses of anticoagulant), and fails to dissolve the existing thrombi. It was shown further that introduction into the circulation bed of anticoagulative agents, fibrinolysin and heparin, intensifies the fibrinolytic activity of the blood and facilitates the dissolution of intravascular blood clots.

Experimental and clinical study of the natural enzyme of the human blood—fibrinolysin has opened up wide opportunities for the treatment of thromboembolic diseases (G. V. Andreyenko, E. I. Chazov; E. I. Chazov, G. V. Andreyenko; Cliffton; Moser).

Another way to increase the fibrinolytic activity of the blood and promote the lysis of thrombi is an intravenous injection of profibrinolysin activators so as to invigorate the patient's own fibrinolytic system, which is often depressed. Several clinics abroad use for this purpose streptokinase, an enzyme recovered from the culture-fluid of β-haemolytic streptococci. However,

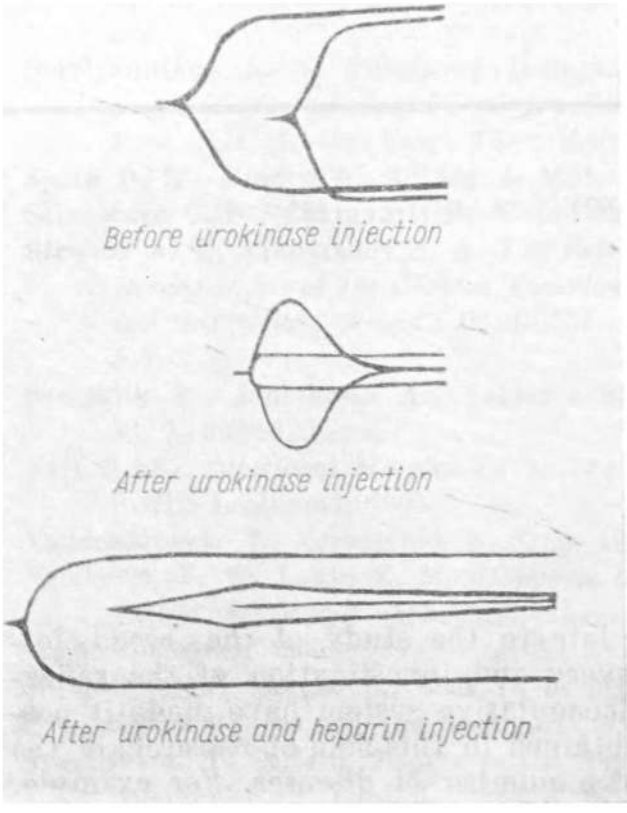

Fig. 1. Thromboelastograms of rabbits with experimental atherosclerosis before and after injection of urokinase with heparin

the high pyrogenicity and antigenic properties of streptokinase preparations limit the scope of their application in clinical practice.

A highly promising agent for the treatment of thromboses is urokinase which has no unwanted properties. Urokinase is a proteolytic enzyme isolated from human urine, readily soluble in water, resistant to heating up to 66 °C and changes of pH within a range from 4 to 10.

Activation of profibrinolysin with urokinase is an enzymatic reaction with an optimum pH equal to 9. Urokinase was for the first time discovered in the human urine by Astrup and Sterndorf (1952) and Kaulla and isolated in pure form by Ploug and Kjeldyaard (1956). Mohler and others showed that urokinase is capable of activating profibrinolysin in a large number of animal species and man.

G. V. Andreyenko and S. M. Strukova injected to albino rats urokinase isolated from human urine and observed a considerable increase in the fibrinolytic activity of the blood caused by a 2 mg dose of the preparation.

Fig. 2. Microphotograph. A thrombus in a rabbit ear vessel induced by injection of thrombin into an isolated region. Magnification 100 times

The present article describes the results of an experimental study of the action of urokinase on the fibrinolytic activity of the blood, and on the dissolution of thrombi in the vessels of rabbits, as well as the preventive effects of this preparation on thrombus formation.

The urokinase preparation used in the experiments had been isolated by a member of our research group from human urine by means of modified methods of Sqouris, Ploug and Celander. The activity of the preparation ranged from 2,000 to 6,000 U per mg of protein. All experiments were made on rabbits with experimental atherosclerosis induced by keeping them on a cholesterol diet during 10 weeks.

Intravenous injection of 50 mg of urokinase in a 20 ml dose of physiological saline to six rabbits provoked in 30-45 minutes' time an increase in the fibrinolytic activity of the blood, which was recorded by means of a thromboelastograph. A considerably greater effect was noted after a simultaneous injection of urokinase in combination with small doses of heparin (40-50 U per rabbit)

Fig. 3. Microphotograph. The free lumen of a rabbit ear vessel after injection of urokinase. The thrombus has dissolved. Magnification 100 times

(Fig. 1). A high-degree fibrinolytic activity was also observed after a repeated injection of urokinase on the next day.

In the second series of experiments, attempts were made to destroy the newly-formed thrombi by injection of urokinase. Thrombosis was induced in the marginal vein of the rabbit ear by injection of 0.2 ml of thrombin solution with 12 sec activity heated to 37 °C into a 1.5 cm portion of the vessel compressed between soft clamps. The injection of urokinase in combination with small doses of heparin led to destruction of the thrombus within 2-2$^1/_2$ hours, which was confirmed by histological and x-ray examination using a contrast stain (Figs. 2, 3).

For a study of the preventive effect of the preparation, six rabbits with experimental atherosclerosis were during a period of 5 days given injections of 30 mg of urokinase dissolved in 20 ml of physiological saline and followed in 30 minutes' time by 3 ml doses of thrombin solution with 12 sec activity and 0.25 ml of pituitrin. As was shown earlier, injection of thrombin during

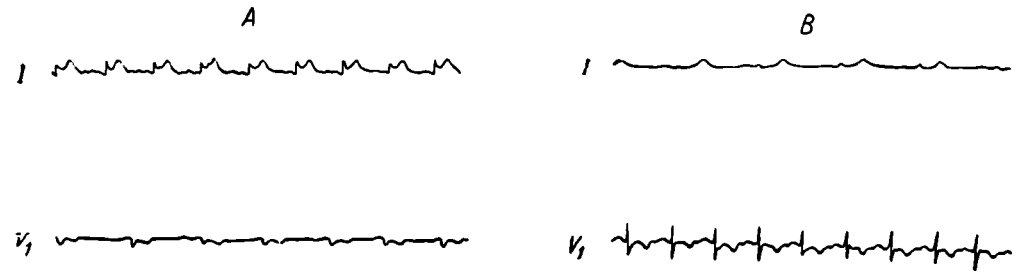

Fig. 4. Elastogram of a rabbit

A—after administration of thrombin with pituitrin; *B*—after injection of urokinase and subsequent administration of thrombin with pituitrin

a spasm caused by pituitrin provokes an acute coronary insufficiency (E. I. Chazov). Preliminary injection of urokinase prevented the development of the above-mentioned circulatory disorders in rabbits (Fig. 4). Histological study of the myocardium and vessels in the animals showed full patency of the vessels, while the myocardium was found to have no necrotic areas usually seen upon injection of thrombin and pituitrin to atherosclerotic rabbits.

CONCLUSION

The experimental findings indicate that a urokinase preparation injected intravenously to atherosclerotic rabbits tends to intensify the fibrinolytic activity of the blood, resolution of experimental thrombi and prevents the development of acute coronary insufficiency.

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FIBRINOLYSIN TREATMENT OF MYOCARDIAL INFARCTION

T. L. KOZHEVNIKOVA, G. V. ANDREYENKO

The problem of treatment of an acute disturbance of the coronary circulation has hitherto been one of the most urgent problems of internal medicine.

In view of the fact that the development of myocardial infarction is in 50-75 per cent of cases accompanied by coronary thrombosis, the investigators directed their efforts to finding remedies preventing or limiting thrombosis. The advent of indirect-action anticoagulants gave medicine the first opportunity to influence the process of blood coagulation. The discovery of heparin has given the physician a valuable remedy—a natural anticoagulant blocking thrombin action on fibrinogen and thereby interfering with the development of thrombosis.

One of the achievements made during the past few years is the therapeutic application of fibrinolysin—a natural enzyme of the human blood capable of dissolving fibrin filaments.

Prof. B. A. Kudryashov and his collaborators attribute the origination of thrombosis to a functional failure of the physiological anticoagulative system. The agents of this system consisting of a number of natural anticoagulants include heparin and activators of profibrinolysin which turn the latter into an active fibrinolysin.

Experimental and clinical studies have shown that the progression of atherosclerotic changes in the vessels is accompanied by reduction of the fibrinolytic activity of the blood (G. G. Bazazyan et al., B. A. Kudryashov et al., E. I. Chazov, T. L. Kozhevnikova, V. M. Panchenko, Astrup, and others).

In 1957 Cliffton and Caroll began to use fibrinolysin for the treatment of thrombosis of various vascular regions.

In 1959, Cliffton reported a successful application of fibrinolysin for coronary thrombosis. The majority of authors unanimously acknowledge the efficacy of fibrinolysin for peripheral arterial and venous thromboses. The possibility of a therapeutic effect of fibrinolysin in myocardial infarction is still a subject for debate.

One group of researchers believe that the use of fibrinolysin leads to an early abatement of pains, normalization of the ECG, and a less marked increase in blood transaminase, which is evidence of limitation of the zone of myocardial infarction and amelioration of its course (Boyles et al., Ruegsegger, Fletcher et al.). Other authors have doubts as to the effectiveness of fibrinolysin therapy of myocardial infarction considering that its possibilities are greatly exaggerated (Richter et al.).

Moser et al., however, as well as Jung and Duckert point to an intensification of the fibrinolytic activity of the blood following an injection of fibrinolysin preparations.

In the Soviet Union, therapy with lysogenic preparations became possible after the production of fibrinolysin by G. V. Andreyenko in 1961. In the same year, fibrinolysin was for the first time applied to 18 patients with thromboses of various vascular regions, among whom 11 had myocardial infarction (E. I. Chazov, G. V. Andreyenko, 1962). The authors noted the high lysogenic capacity of the preparation, particularly when it was used early. Fibrinolysin was administered in combination with heparin, imitating the natural reaction of the anticoagulative system like it happens in a living body endangered by thrombosis. Experience has shown that injection of heparin alone only restricts the progression of thrombosis but fails to dissolve the fibrin filaments.

We have carried out experiments with fibrinolysin treatment of 30 patients with acute myocardial infarction hospitalized at the Institute of Therapy. We succeeded in administering fibrinolysin within 24 hours after the onset of the disease to 24 patients, while to the other 6 patients the preparation was given on the second day.

In the majority of patients the diagnosis of myocardial infarction was confirmed by electrocardiography. In some cases, however, no distinct ECG changes were recorded since the treatment had been started early.

In addition to the clinical and instrumental examination of patients, which was required for the diagnosis, we determined the fibrinolytic activity of the blood before and after fibrinolysin administration and undertook thromboelastography. A. A. Nekrasova measured the level of blood transaminase.

Fibrinolysin dissolved in a physiological saline solution was injected by drops during 3-4 hours in a dose of 16,000-30,000 U in combination with 10,000-15,000 U of heparin. Upon completion of transfusion, heparin was injected intramuscularly in a daily dose of 25,000-30,000 U, the clotting time being checked.

Eighteen patients were given a single dose of fibrinolysin. In 12 cases, fibrinolysin transfusion was repeated on the next day in a dose of 9,000-15,000 U. Upon repeated fibrinolysin administration the dosage of heparin was lessened to 5,000 U; continued injection of heparin depended on blood-clotting indices. Later, the patients were given indirect-action anticoagulants.

The best effect was produced by an early application of fibrinolysin, especially by its administration within the first hours after the onset of an anginose attack.

In patients with myocardial infarction a marked analgetic effect of injected fibrinolysin was noted. Even in those cases where intravenous injection of morphine failed to relieve pains, administration of fibrinolysin resulted in aleviation of pain in 25-30 minutes after the beginning of transfusion. Many of the patients became calm and fell asleep during the administration of the preparation.

A follow-up study of these patients revealed the absence of painful sensations in the heart region throughout the period of hospital treatment in 19 patients; 9 patients sometimes felt pain in the heart region, which was quickly relieved with validol or nitroglycerin. Only 2 patients had repeated intense pains in the heart region following fibrinolysin administration. One of these patients had extensive focal changes in the myocardium; treatment was started on the 2nd day and proved futile. In another case, a patient developed postinfarction pericarditis which we believe to be responsible for the absence of an analgetic effect upon administration of fibrinolysin.

Drawing a comparison between a group of patients with an identically severe disease and the patients under our observation who had not been given anticoagulant treatment it was found that it was early administration of fibrinolysin with heparin that accounted for the relatively mild course of the disease. This was manifested by the absence of ECG changes characteristic of myocardial infarction (in 8 persons out of 30) or by a faster restoration of the earlier recorded ECG changes pointing to derangements of the coronary circulation (in 18 persons). Four patients out of thirty, however, showed no positive ECG changes following fibrinolysin administration.

Nineteen patients treated with fibrinolysin showed no temperature reaction to the onset of myocardial infarction, and in 15 patients no characteristic changes were noted in the clinical pic-

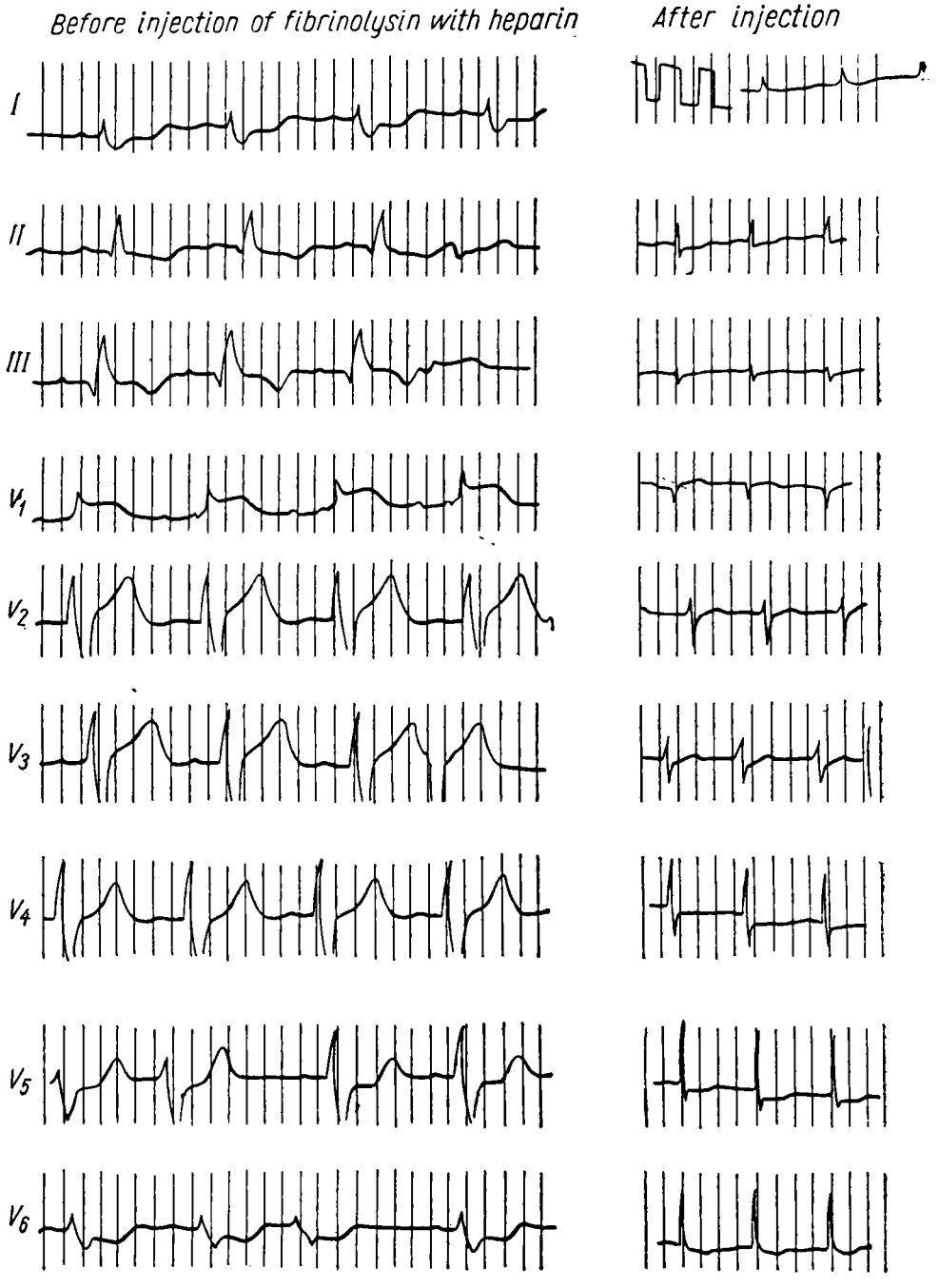

Fig. 1. Electrocardiograms of patient B. before and after fibrinolysin and heparin treatment

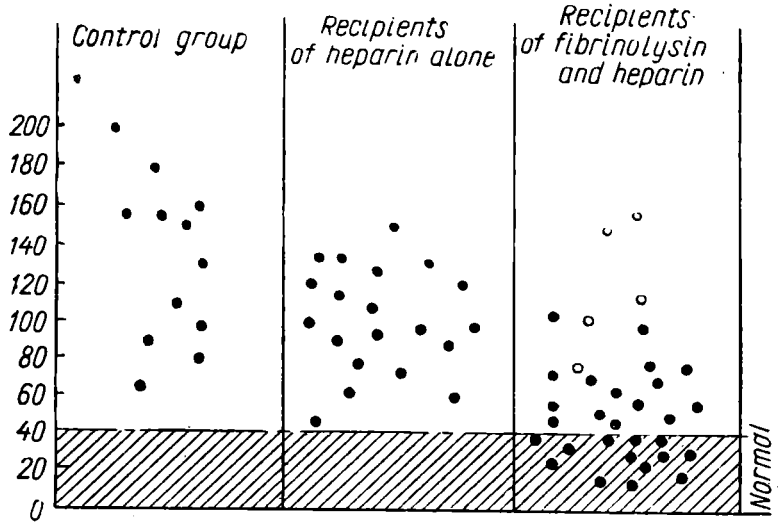

Fig. 2. The level of blood transaminase during myocardial infarction in patients subdivided into three groups: (1) those not given anticoagulants; (2) recipients of heparin; and (3) recipients of fibrinolysin in combination with heparin

On axis of ordinate—transaminase content in U; shaded strip—normal transaminase content; black circles—transaminase content within 24 hours of treatment; light circles—transaminase content in patients given anticoagulants on 2nd day of disease

ture of the blood. A fatal outcome occurred only in one case in which treatment was undertaken on the 2nd day of the disease.

Fig. 1 shows the electrocardiogram of patient B. At left is the curve recorded before administration of fibrinolysin with heparin, at right is the curve recorded immediately upon injection of the preparations. The marked positive changes in the coronary complex are distinctly seen in all leads.

The objective index of the degree of heart muscle necrosis is the level of blood transaminase. Fig. 2 shows the level of blood transaminase in three groups of patients. The 1st column indicates the transaminase level in the group of patients not given anticoagulant treatment, the 2nd column the level of transaminase in patients treated exclusively with heparin, and the 3rd column shows the level of this enzyme in the blood of patients treated with fibrinolysin and heparin in combination. It is seen clearly that the greatest increase in the transaminase level takes place in the first (control) group of patients, and the smallest increase is noted in persons treated with fibrinolysin and heparin during the first 24 hours. These indices apparently point to lim-

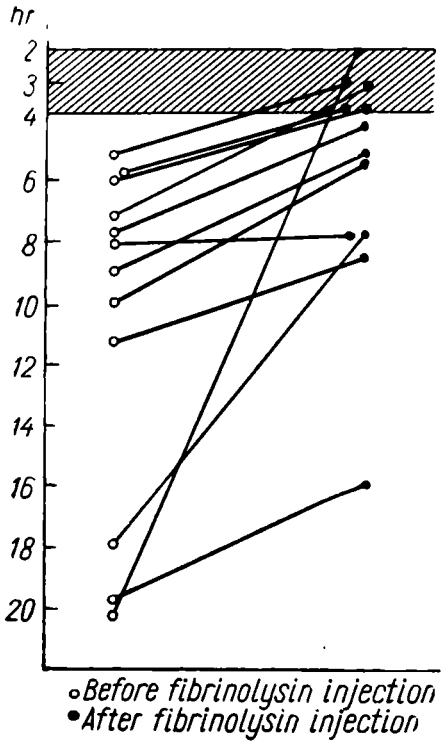

Fig. 3. Fibrinolytic activity of the blood before and after fibrinolysin injection.

On axis of ordinate is indicated fibrinolysis time in hours; shaded strip—normal level of fibrinolysin; light circles—fibrinolysis before fibrinolysin injection; black circles—fibrinolysis after fibrinolysin injection

itation of the necrotic area in the myocardium in the case of the above-mentioned therapy.

Case history No. 2379 is presented for illustration.

Patient B., 69, a pensioner. Admitted to the clinic on 28 September, 1962, with complaints of heavy pains behind the sternum, debility, sensation of air deficiency. Status anginosus developed 6 hours before admission to the clinic. Administration of anaesthetic and vasodilating drugs relieved acute pains which had lasted $3^1/_2$ hours, though sensation of heaviness and compression behind the sternum, debility and fear persisted.

At the time of admission the patient's condition was grave. The skin was pale, cold perspiration. The pulse was soft, 100-120 beats per min, with frequent extrasystoles which later transformed into fibrillation. The arterial pressure 90/60 mm Hg. The heart borders were expanded leftwards, the cardiac sounds muffled. Blood analysis revealed leukocytosis, fast ESR —up to 15-38 mm/hr. ECG indicated focal changes of the myocardium in the anterolateral wall of the left ventricle.

Fibrinolysin was administered twice: on the 1st day in a dose of 16,000 U in combination with 10,000 U of heparin; on the next day in a dose of 12,000 U combined with 5,000 U of heparin. In the intervals between repeated fibrinolysin transfusions heparin was injected intramuscularly the clotting time being checked. Cardiac drugs, oxygen, and symptomatic remedies were used.

Fibrinolysin completely eliminated pain, and the patient dozed off during its administration. Tachycardia, however, persisted at the same rate. Cardiac fibrillation continued during three days, which was followed by restoration of the sinus rhythm. The subsequent course of the disease was quite uneventful. No painful sensations were felt behind the sternum

through the entire observation period, the pulse-rate slowed down, the arterial pressure returned to normal, electrocardiography revealed normalization of curves following fibrinolysin injection. The patient was discharged in a month's time in a satisfactory condition.

A study of the influence of fibrinolysin on the biochemical indices of blood-clotting has shown that the fibrinolytic activity of the blood noticeably increases after injection of the preparation.

This is shown in Fig. 3 which graphically illustrates the degree of intensification of the fibrinolytic activity of the blood after fibrinolysin administration.

The most adequate characteristics of the blood-clotting process may be obtained by means of thromboelastography. We have succeeded in revealing three types of the most characteristic thromboelastographic changes as a result of fibrinolysin administration (Fig. 4).

Changes of the 1st type are characteristic of increased fibrinolysis. Changes of the 2nd type are evidence of a sharp slowing of the blood-clotting process and looseness of the fibrin clot. The third type of changes shows complete absence of blood clotting throughout the period of investigations.

It was established thromboelastographically that in some patients the blood coagulability returns to the initial (increased) level 24 hours after the first fibrinolysin administration. This was the reason for a repeated fibrinolysin injection in 12 patients. Their treatment proved quite satisfactory.

It should be noted that when using the above-mentioned method for administering the preparation we rarely observed untoward effects. One patient vomited coffee grounds on one occasion.

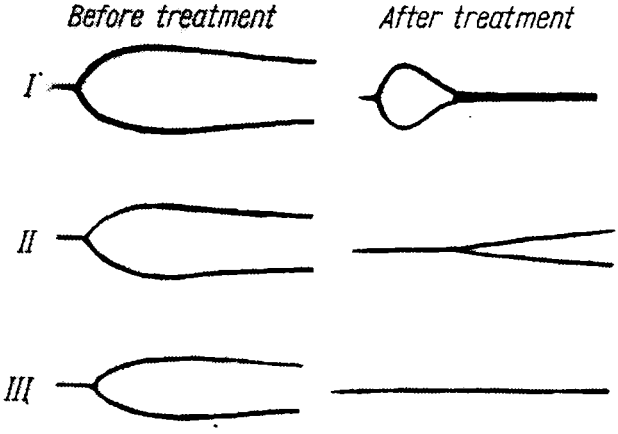

Fig. 4. Thromboelastograms. At left—before fibrinolysin treatment; at right—after fibrinolysin treatment

Four patients were noted to have insignificant rigor which was stopped early by the application of heat and promedol injections.

CONCLUSION

The application of fibrinolysin in combination with heparin is a hopeful new method of therapy of acute myocardial infarction, particularly in its early stages. Fibrinolysin treatment produces a marked analgetic effect, making the disease run a milder course.

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DIAGNOSTIC VALUE OF DETERMINATION OF DNA DECAY PRODUCTS AND SULPHOHYDRYL GROUPS IN THE BLOOD SERUM DURING MYOCARDIAL INFARCTION

L. T. LYSENKO, B. S. SUKHAREVA

Differential diagnosis between angina pectoris and myocardial infarction is often difficult in cases where the ECG shows no changes characteristic of the latter disease.

The methods suggested during the past few years for investigation of the activity of certain enzymes: transaminase (La Due, et al.), aldolase (Volk a. Losner), lactic dehydrogenase (White), etc., as diagnostic tests in myocardial infarction reveal changes in the blood serum in the early days of the disease. We thought it was helpful to search for other biochemical indices capable of facilitating the diagnosis of myocardial infarction. We chose the determination of low-molecular oxygen-soluble derivatives of desoxyribose and sulphohydryl groups of the blood serum.

The search for desoxyribose derivatives in the blood was based on the assumption that it was possible for DNA decay products to leave the cell nuclei of the necrotized portion of the heart muscle and enter the blood stream. The histochemical studies carried out by A. K. Ageyev who traced in the process of tissue death DNA diffusion from the cell nuclei into the blood vessels along the periphery of the necrotized area are evidence in favour of this possibility. The acid soluble desoxyribose derivatives were determined after the classical Dichet's method with diphenylamine. Spectrophotometric study of the experimental solution was carried out against a standard consisting of the same components taken in the same proportions as those of the experimental solution except for diphenylamine. Quantitative estimation of desoxyribose derivatives was made according to calibration curves plotted with the use of a serum to which DNA had

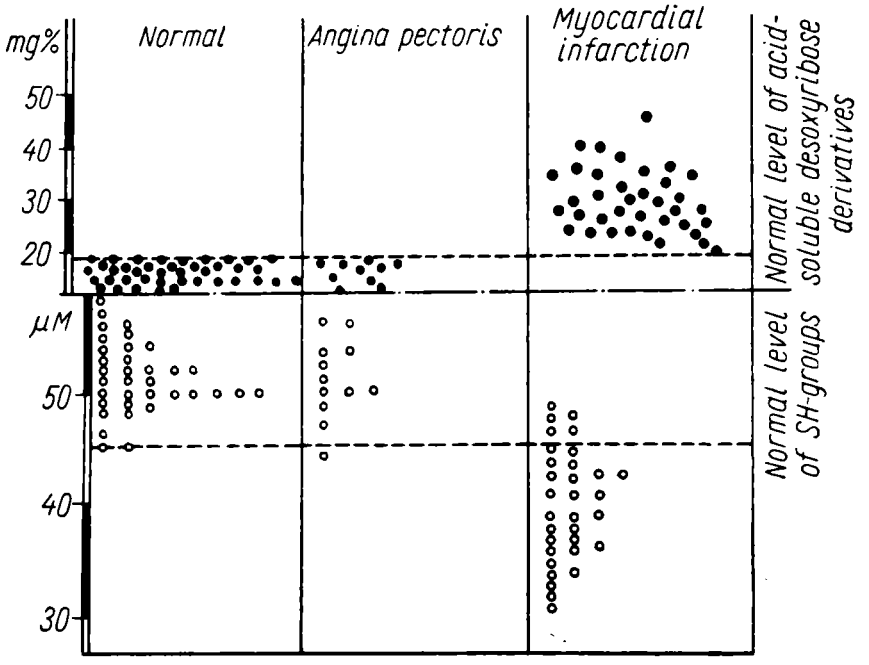


Fig. 1. The level of acid-soluble desoxyribose derivatives and SH groups in the blood serum of healthy persons, in patients with coronary atherosclerosis and attacks of angina pectoris and in patients with acute myocardial infarction.

On axis of ordinate is indicated the level of desoxyribose derivatives in mg per cent and SH groups in μM/100 ml of blood serum. Black dots indicate acid-soluble desoxyribose derivatives; circles indicate SH groups of the blood serum

been added. The results of the investigations were expressed in mg per cent units of decayed DNA.

The precondition for investigating the sulphohydryl groups of the blood serum was the findings made by a number of authors who showed the high reactive capacity of these groups, their participation in the fixation of various kinds of substances (Barron). The sulphohydryl groups of the blood serum were determined by the method of ammeter titration after Kolthoff and Harris with the use of silver nitrate. The data obtained were expressed in micromoles per 100 ml of serum.

The level of desoxyribose compounds was studied in 40 healthy persons ranging in age from 20 to 56 years, in 10 patients with coronary atherosclerosis and attacks of angina pectoris, and in 37 patients with acute myocardial infarction. As is seen from Fig. 1 the level of acid-soluble compounds of desoxyribose in the blood serum of healthy persons was 12-18 mg per cent, in patients with coronary atherosclerosis and attacks of angina pectoris the level of desoxyribose compounds was within the same limits as in healthy persons. In patients with acute myocardial infarction the

level of acid-soluble desoxyribose derivatives proved to be raised up to 50 mg per cent. Investigations of the sulphohydryl groups of the blood serum were carried out in 35 healthy persons, 12 patients with coronary atherosclerosis and attacks of angina pectoris and 32 patients with acute myocardial infarction (Fig. 1). Normally, the level of the sulphohydryl groups of the blood serum varied from 45 to 58 μM per 100 ml of serum. In 11 patients with coronary atherosclerosis and attacks of angina pectoris the level of the sulphohydryl groups of the blood serum was within normal limits, and only in one patient it was decreased somewhat. Reduction of the level of sulphohydryl groups in the blood serum down to 30 μM per 100 ml of serum was discovered only in 22 out of 27 patients with acute myocardial infarction; in 5 patients no changes were found.

A distinct correlation has been established between both indices in the course of the disease. Several examples follow.

Patient T., male, aged 46 years. Since December, 1961, troubled by brief, pressing retrosternal pains in walking. On the night of February 10, 1962, suffered a very intense anginose attack which lasted several hours; following a promedol injection the pains abated somewhat. On February 10, the patient was admitted to the Institute of Therapy. In the evening, fever rose to 37.2°C, in the later days to 38°C. The leukocyte count was 16,900, ESR—28 mm/hr. The ECG showed a picture of infarction of the anterolateral wall of the left ventricle. Since February 11 the pains were absent. The further course of the disease was uneventful.

As is seen in Fig. 2*A*, the level of the acid-soluble desoxyribose derivatives investigated on the 5th day after the onset of pain attack proved high—33.6 mg per cent, on the 10th day it reduced somewhat—24.8 mg per cent; the amount of acid-soluble desoxyribose derivatives studied on the 19th day of the disease proved normal. The level of the sulphohydryl groups of the blood serum on the 5th day of the disease was found to be markedly lowered—35.6 μM per 100 ml of serum, on the 10th day it remained at the same level—36 μM per 100 ml of serum. On the 19th day of the disease the level of the sulphohydryl groups increased—41.5 μM, on the 30th day it returned to normal—50 μM. Later, both indices remained throughout the period of hospital treatment.

Analogous changes in the level of the derivatives of desoxyribose and sulphohydryl groups of the blood serum were observed in patient K., male, aged 57 years, with an extensive myocardial infarction of the anterolateral wall of the left ventricle involving the interventricular septum: on February 18 the patient sustained a severe attack of angina pectoris accompanied by a collaptoid state. In the evening on the same day fever rose to 37.1°C, and in the later days to 38.1°C. The leukocyte count was 12,600, ESR—30 mm/hr. The ECG showed signs of extensive infarction in the anteroseptal, apical regions and the lateral wall of the left ventricle. There were acute symptoms of cardiac insufficiency: tachycardia, and congestion in the lungs and liver.

Since February 21, anginose attacks ceased, and symptoms of cardiac insufficiency disappeared in a fortnight. The ECG showed positive changes. The leukocyte count decreased to 4,500, ESR to 17 mm/hr.

On the 3rd day of the disease, the level of acid-soluble compounds of blood serum desoxyribose remained increased—26.4 mg per cent (Fig. 2*B*).

Fig. 2.

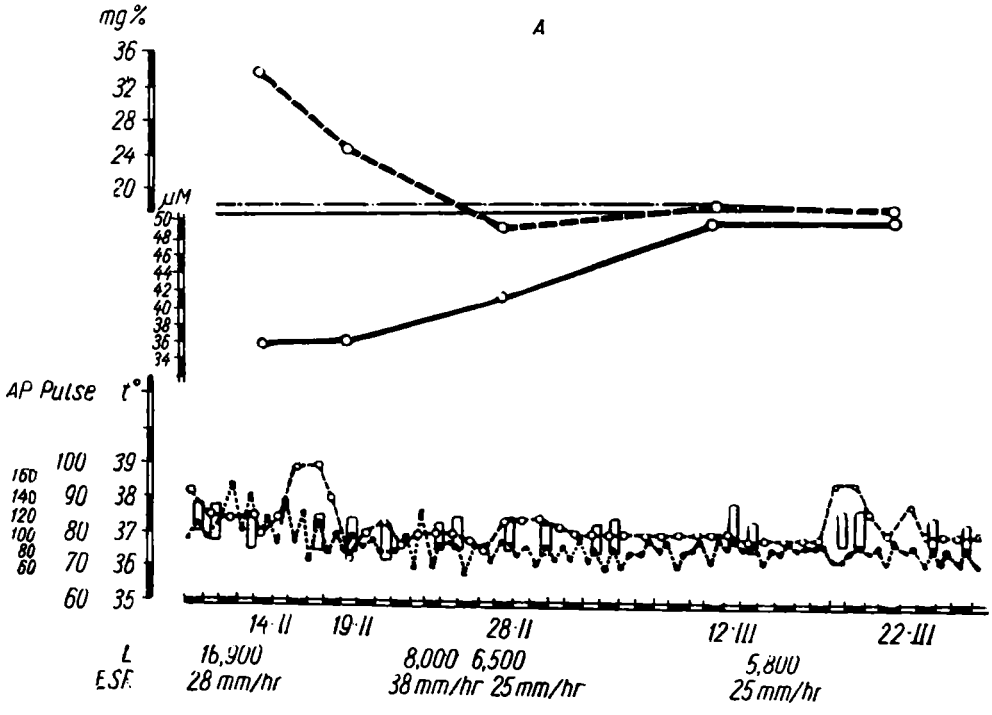
A—level of acid-soluble desoxyribose compounds and SH groups in the blood serum of patient T. On axis of ordinate are indicated the level of desoxyribose derivatives in mg per cent, SH groups in μM/100 ml of the blood serum, body temperature, the pulse rate and arterial pressure. On axis of abscissa are indicated days of disease. Circles connected by the intermittent line indicate the level of acid-soluble desoxyribose derivatives in the blood serum; circles connected by the continuous line indicate the level of SH groups in the blood serum; the intermittent-dotted line indicates the upper limit of acid-soluble desoxyribose derivatives in the blood serum under normal conditions; the thin continuous horizontal line indicates the average normal level of SH groups in the blood serum; dots connected by the dotted line indicate body temperature; circles connected by the thin intermittent line indicate the pulse rate; columns indicate the arterial pressure;

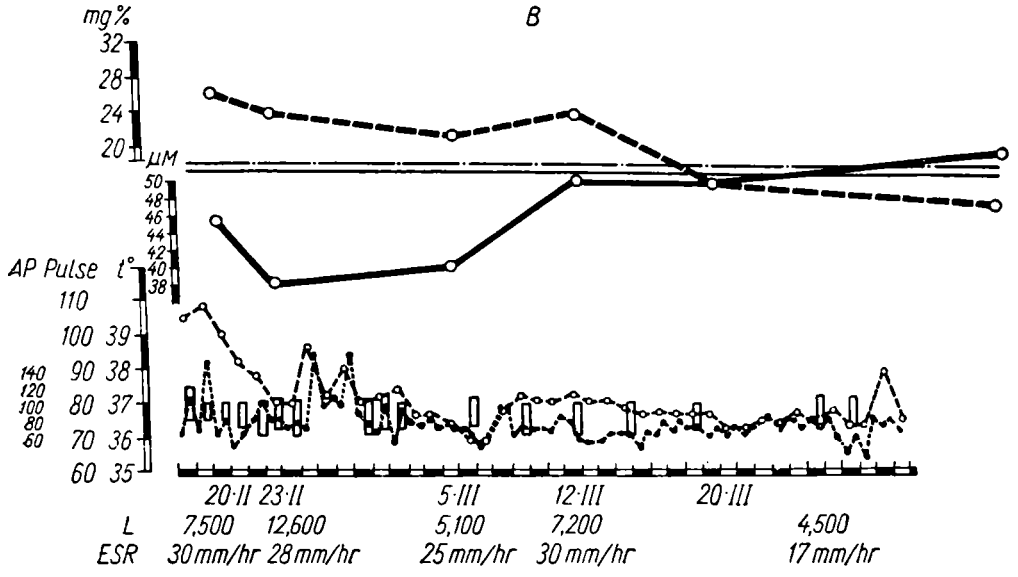
B—level of acid-soluble desoxyribose compounds and SH groups in the blood serum of patient K. Designations are the same as in Fig. 2*A*;

C—level of acid-soluble desoxyribose derivatives and SH groups in the blood serum of patient F. Designations are the same as in Fig. 2*A*. Arrow indicates relapsing myocardial infarction;

D—level of acid-soluble desoxyribose derivatives and SH groups in the blood serum of patient B. Designations are the same as in Fig. 2*A*. Arrow indicates relapsing myocardial infarction;

E—level of the acid-soluble desoxyribose derivatives and SH groups in the blood serum of patient M. Designations are the same as in Fig. 2*A*.

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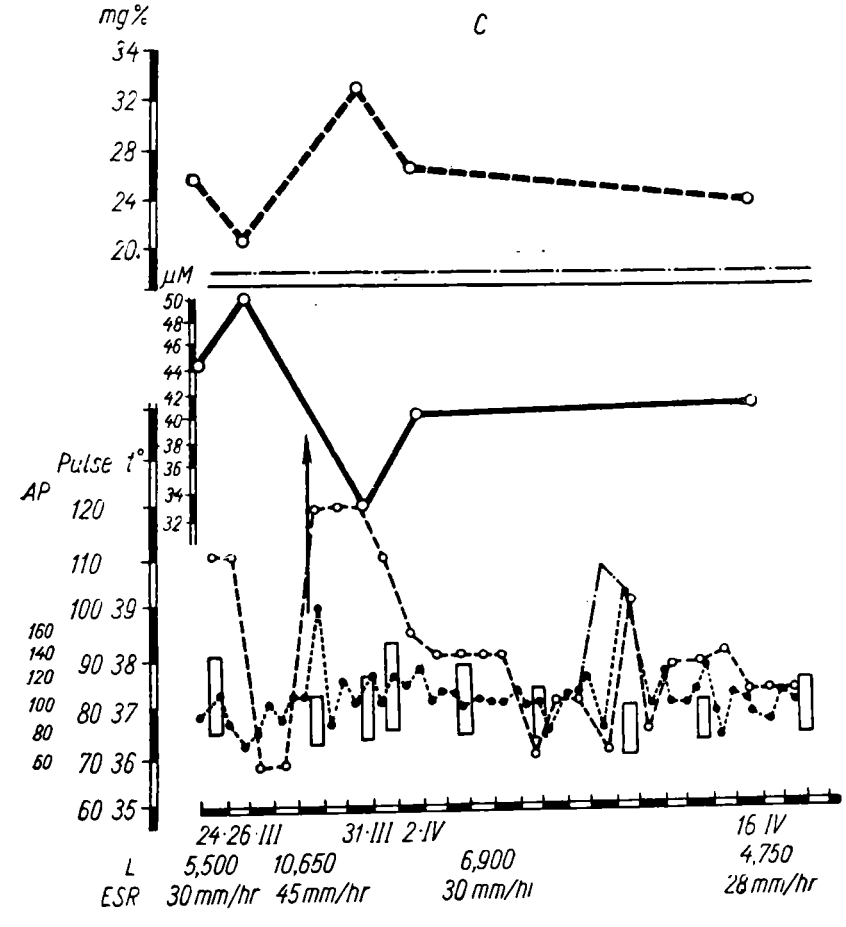
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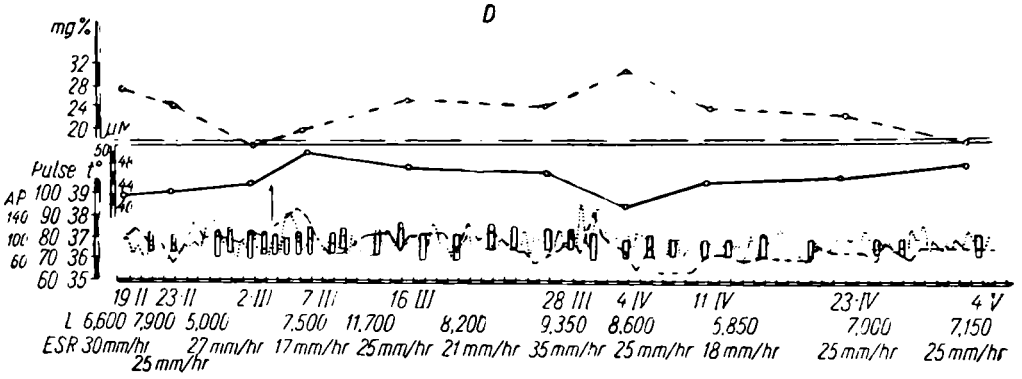
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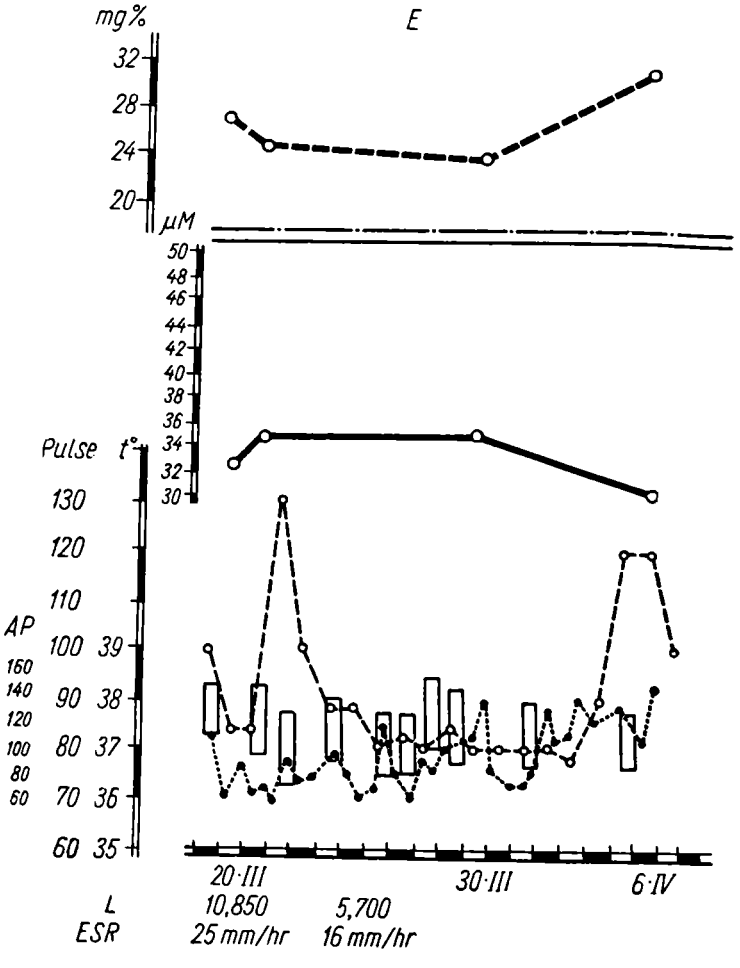
25 mm/hr


mg%
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20 III 30 III 6 IV
L 10,850 5,700
ESR 25 mm/hr 16 mm/hr

The desoxyribose derivatives returned to normal on the 31st day of the disease. On the 3rd day of the disease the level of the sulphohydryl groups of the blood serum was at the lowest normal level—45 μ M, on the 6th day it reduced to 38 μM; on the 15th day the amount of sulphohydryl groups of the serum remained decreased—40 μM, and on the 23rd day of the disease became normal—50 μM per 100 ml of blood serum. Later, it remained within normal limits (49 μM on the 31st day and 54 μM on the 54th day of the disease).

Thus, the increase in the level of the blood serum desoxyribose derivatives and reduction of the blood serum sulphohydryl groups occurred more often on the 2nd day of myocardial infarction. Changes of both indices persisted during 2-3 weeks and sometimes longer, depending on the course of the disease. Repeated changes in both indices were observed during myocardial infarction with a recidivating course (Fig. 2, *C*,*D*,*E*).

Patient F., female, aged 66 years (Fig. 2*C*). Suffered from hypertension since 1946. Admitted to the Institute of Therapy on 23 March, 1962, for myocardial infarction on the 10th day of the disease. ECG showed signs of infarction in the posterior wall of the left ventricle. The leukocyte count was 5,500, ESR—30 mm/hr. On March 28 the patient suffered a repeated prolonged attack of pain. In the evening on the same day fever rose to 38.2°C and remained subfebrile during the subsequent days. The leukocyte count rose to 10,650, ESR to 45 mm/hr. The diagnosis was one of relapsing myocardial infarction which according to ECG data was confined to the anterior wall of the left ventricle.

The acid-soluble desoxyribose compounds investigated in the patient's blood serum on the 11th day of myocardial infarction had not yet returned to normal, and were equal to 25.6 mg per cent. On the 13th day of the disease they reduced to 21.6 mg per cent. Investigation on the 3rd day after relapsing myocardial infarction showed a sharp increase in the level of desoxyribose derivatives in the blood serum to 33.2 mg per cent. The level of the blood serum sulphohydryl groups following the first myocardial infarction returned to normal; on the 3rd day after repeated myocardial infarction it was found to be sharply decreased.

A repeated change in the level of desoxyribose derivatives and the blood serum sulphohydryl groups in response to relapsing myocardial infarction was also observed in female patient B., aged 65 years (Fig. 2*D*).

The patient was admitted to the Institute of Therapy for myocardial infarction of the anterolateral wall of the left ventricle and septum on 16 February, 1962, on the 3rd day of the disease. Nineteen days after the first myocardial infarction the patient developed an anginose state which could not be aborted during 6 hours. On the next day fever rose to 38°C. The leukocyte count rose from 5,000 to 11,700. ECG showed signs of fresh myocardial infarction in the anteroseptal, apical and lateral regions of the left ventricle. After relapsing myocardial infarction the normalized acid-soluble desoxyribose derivatives and the sulphohydryl groups of the blood serum again underwent a change, acid-soluble desoxyribose compounds 28.8 mg per cent, sulphohydryl groups of blood serum 40 μM per 100 ml

of serum. Both indices returned to normal only two months after relapsing myocardial infarction.

It is interesting to see the dynamics of changes in the desoxyribose derivatives and sulphohydryl groups of the blood serum in patient M., male, aged 68 years, in whom relapsing myocardial infarction had not been identified during life (Fig. 2*E*).

The patient was admitted to the Institute of Therapy for acute myocardial infarction. Repeated ECG findings suggested focal changes of an ischaemic character in the posterior wall of the left ventricle in the presence of a chronic coronary insufficiency. The patient's condition was unvariably grave. Progression of the signs of cardiac insufficiency was noted mainly in the pulmonary circulation. From time to time disturbances in the respiratory rhythm after the Cheyne-Stokes type were noted. During confinement in hospital no repeated attacks of pain were registered. The ECG taken on the eve and on the day of the patient's death showed no new changes. The acid-soluble desoxyribose compounds investigated one day before death proved markedly increased, and the sulphohydryl groups of the blood serum showed a sharp reduction as compared to the earlier level of these compounds in the blood serum.

In addition to the old scar of the anterior wall, autopsy revealed an extensive new three-day-old myocardial infarction in the posterior and lateral walls of the left ventricle, the apex and the posterior part of the interventricular septum.

It follows from the above examples that the level of the low-molecular desoxyribose derivatives and the sulphohydryl groups of the blood serum in patients with acute myocardial infarction reflects the course of the disease.

Fig. 3 demonstrates the relationship of the degree of increase in the low-molecular desoxyribose derivatives and reduction of the sulphohydryl groups of the blood serum to the necrotic focus area. Extensive myocardial infarctions were accompanied by larger deviations of both indices than were microfocal necroses. In the group of patients with extensive myocardial infarction the average level of acid-soluble desoxyribose derivatives was 31.54 mg per cent, the average level of the sulphohydryl groups of the blood serum was 38 μM per 100 ml of serum, and in the group of patients with microfocal lesions the average values of both indices were 23.5 mg per cent and 43 μM per 100 ml of serum respectively.

Correlation between the changes in the low-molecular desoxyribose derivatives and reduction of the sulphohydryl groups of the blood serum which we have noted in isolated clinical cases may also indicate that the sulphohydryl groups of the blood serum bind the decay products (in particular, desoxyribose derivatives) supplied to the blood from the necrotized portions of the heart muscle.

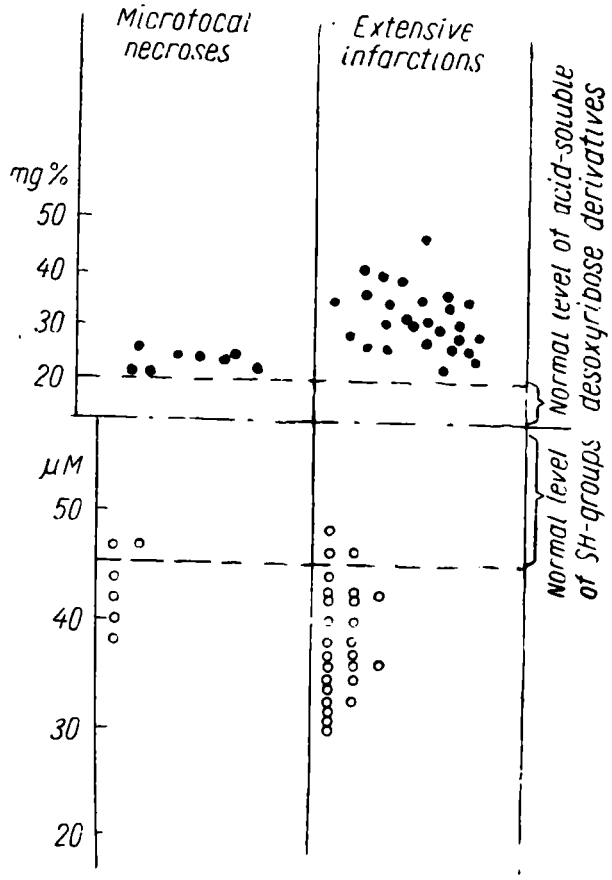

Fig. 3. The level of acid-soluble desoxyribose derivatives and SH groups of the blood serum in microfocal and extensive myocardial infarctions. Same designations as in Fig. 1

It is beyond doubt that the level of desoxyribose derivatives in the blood serum is increased in all kinds of necrotic and inflammatory processes occurring in the body. Reduction of the level of the blood serum sulphohydryl groups takes place not only in myocardial infarction but also in a number of other diseases. The fact, however, that the level of desoxyribose derivatives and the sulphohydryl groups of the blood serum in patients with coronary atherosclerosis and attacks of angina pectoris remains within normal limits, while in myocardial infarction a change in these indices is observed throughout the acute period of disease, makes it possible to use these methods for a differential diagnosis between myocardial infarction and angina pectoris and to follow the course of myocardial infarction.

CONCLUSIONS

1. In myocardial infarction, the level of the low-molecular desoxyribose derivatives in the blood serum is increased usually on the 2nd day of the disease and the level of the sulphohydryl groups of the blood serum is reduced simultaneously or with one

or two days' delay. Changes in both indices persist during 2-3 weeks, after which they return to normal.

During myocardial infarction with a recidivating course a repeated change in the level of desoxyribose derivatives and the sulphohydryl groups of the blood serum takes place.

2. The level of the low-molecular desoxyribose derivatives and the sulphohydryl groups of the blood serum in patients with coronary atherosclerosis combined with angina pectoris attacks but without myocardial infarction remains within normal limits.

3. Investigations of the low-molecular desoxyribose derivatives and the sulphohydryl groups of the blood serum may be used for a differential diagnosis between myocardial infarction and angina pectoris.

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THE MECHANISM OF CARDIAC FAILURE IN ACUTE MYOCARDIAL INFARCTION

Y. T. PUSHKAR, N. M. MUKHARLYAMOV, E. I. CHAZOV

The study of the contractile function of the heart in the acute period of myocardial infarction is very important for clinical practice. The opportunities for investigating the haemodynamics at the beginning of the disease are usually considerably limited as a result of acute circulatory insufficiency. Therefore, the circulatory disturbances due to decompensation of the myocardium are difficult to reveal at the beginning of the disease by the conventional clinical examination.

It is known that in the acute period of myocardial infarction haemodynamic changes are characterized by a reduction of the minute volume and the arterial pressure, the restoration of which requires an intensified activity of the myocardium (Wiggers). An important place among the causes of death in myocardial infarction belongs to progressive circulatory insufficiency, whose mechanism is little known so far. The intensity of the contractile function of the heart is characterized by the tension developed in the ventricular myocardium during a systole (Rushmer, V. V. Parin).

The average intensity of myocardial tension during the cardiac cycle is expressed by this formula: $T_{sp.} = \frac{P \cdot r}{2} \cdot 1332 \cdot 10^{-6}$ (dyne/cm), where $T_{sp.}$ is the average intensity of myocardial tension during the cardiac cycle expressed in dyne/cm, P—the average systolic pressure in the ventricle in millimetres Hg, r—the ventricle radius in the phase of isometric tension in centimetres, $1332 \cdot 10^{-6}$—the coefficient for conversion of pressure indices expressed in millimetres Hg into dyne/cm^2.

The summary per-minute intensity of myocardial tension in the left ventricle is the product of $T_{sp.}$ multiplied by the number of heart contractions per min.

It follows from the formula that with an increase in the ventricular radius in patients with myocardial infarction as well as in those with cardiac aneurysms (V. L. Karpman et al.), the normal pressure in the heart cavities may be attained through an intensified contraction of the intact parts of the myocardium. The developing compensatory hyperfunction of the myocardium can at the same time ensure an adequate minute volume; later, however, this hyperfunction may result in exhaustion of the myocardium. An especially unfavourable factor is the shortening of the expulsion phase. This results in an impairment of the cardiac function and decompensation.

This new interpretation of the haemodynamic disturbances as well as the comparison drawn between the minute volume and the cardiac contraction phases enable one to get a better idea of the mechanism of cardiac failure in patients with myocardial infarction.

The indices reflecting the intensity of the contractile function of the heart and, consequently, the myocardial tension are the duration of the isometric contraction and the height of the systolic arterial pressure. It is supposed that the systolic pressure does not differ from the intraventricular pressure significantly.

A marked prolongation of the isometric contraction and reduction of the systolic pressure point to a decreased intensity of cardiac contractions.

The index of the effective cardiac hyperfunction is the value of the minute blood volume. During reduction of the functional activity of the preserved portions of the myocardium reduction of the minute volume leads to a circulatory insufficiency in spite of the increased intensity of myocardial tension.

For analysis of the contractile function of the heart the time of change in the ventricular form was measured (phase of asynchronous contraction) as well as the isometric contraction phase, the tension phase, the expulsion phase, the protodiastolic period, the duration of the mechanical systole; at the same time, the intrasystolic index ISI ($ISI = \frac{\text{expulsion phase}}{\text{mechanical systole duration}}$) and the haemodynamic index I ($I = \frac{\text{expulsion phase}}{\text{tension phase}}$) were calculated.

Simultaneously, the systolic and minute volumes were determined after Hamilton in Wood's modification and after Broemser—Ranke (physical method). The curve of stain T-1824 dilution was registered by means of an ear recorder. The phase analysis was carried out by Blumberger and Holldack's method taking into account the duration of the protodiastolic period.

We repeatedly examined 38 patients with myocardial infarction, beginning from the early days and sometimes early hours after the onset of an anginose attack. Twenty-two patients were noted to have a milder course, in the other 16 myocardial infarction ran a severe course.

In 14 out of 22 patients with a milder course of the disease in the beginning of the acute period the per minute blood volume varied from 3-3.5 to 5 l; the systolic volume from 24 ml to 60 ml. The haemodynamic index was 1.8-2.5 (normally 2.5-3). No sharp decline of the arterial pressures was noted in patients of this group, except for 3 patients in whom the systolic pressure was below 100 mm Hg. During the first 1-2 days of an anginose attack 6 patients showed a prolongation of the isometric contraction phase accompanied by a decrease in the minute volume to 3-3.5 l as well a shortening of the expulsion phase, a reduction of the haemodynamic index to 1.5-1.8 and of the intrasystolic index to 75 per cent (normal ISI=85-95 per cent). In the other two patients no substantial haemodynamic changes were noted.

In 10 cases out of 16 with a severe course of myocardial infarction signs of a circulatory insufficiency were noted, mainly insufficiency of the left ventricle manifested by short-breath, orthopnoea, cardiac asthma and pulmonary oedema; 4 patients were admitted in a shock condition. In 2 patients the disease took a severe course 3 days after admission to the clinic.

In the group of severely ill patients a considerable decline of both the systolic (down to 16-40 ml) and minute blood volume (down to 2.3 l) was noted in the early period. These changes of the systolic and minute volume in patients with a severe course of myocardial infarction, just as in those with a mild course, corresponded to the data cited in the literature (P. E. Lukomsky and E. M. Tareyev, A. L. Myasnikov, A. V. Vinogradov et al., Plots et al.).

All patients with a low systolic volume showed a considerable reduction of the haemodynamic index (to 1.5 and even to 1) and of the intrasystolic index (to 63 per cent) in connection with prolongation of the tension phase and a shortening of the expulsion phase. The tension phase was prolonged mainly as a result of an increase in the phase of isometric contraction (more than 0.045 sec).

In 2 patients, however, the isometric contraction phase was not prolonged, apparently because of the low diastolic pressure noted in them, owing to which the semilunar valves opened faster at a lower intraventricular pressure.

The systolic arterial pressure was reduced in 4 patients (below 100 mm Hg).

In 12 patients with a severe course of the disease the heart's mechanical action was considerably slackened.

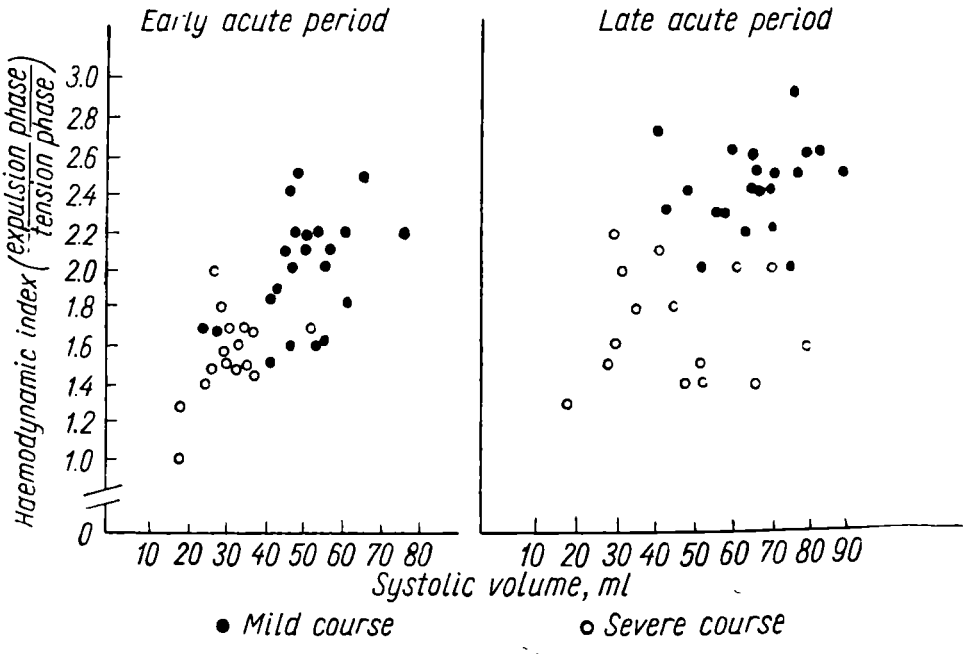

Fig. 1. Correlation between the haemodynamic index and the systolic blood volume in patients with acute myocardial infarction.

On axis of ordinate are indicated values of haemodynamic index; on axis of abscissa—systolic volume (ml)

Under the influence of treatment, the duration of the cardiac contraction phases and the minute volume returned to normal in different stages of myocardial infarction depending on the extension of infarction and the degree of atherosclerotic lesions of the coronary arteries. In extensive infarctions as well as in patients with a marked chronic coronary insufficiency the indices of the contractile functions of the myocardium remained disturbed for a longer time than in those with a less severe affection of the heart (Fig. 1).

As is seen in Fig. 1, patients with a mild course of the disease at the end of the acute period show an increase in the systolic volume and an increase in the haemodynamic index, an improvement of the haemodynamic indices being noted as early as on the 3rd day of the disease. This is also demonstrated by Table 1 which presents the results of examination of patient B., who was admitted in a moderately grave condition 5 hours after the onset of retrosternal pain.

Some of the patients with a severe course of the disease also showed an increase in the systolic volume which was not, as a rule, accompanied by an increase in the haemodynamic index; moreover, even a decline of this index was noted in isolated cases as a result of a further shortening of the expulsion phase.

Table 1

HAEMODYNAMIC INDICES FOR PATIENT B.

Haemodynamic indices	1st day of disease	3rd day of disease	18th day of disease
Isometric contraction phase, sec	0.05	0.04	0.04
Tension phase, sec	0.1	0.1	0.09
Expulsion phase, sec	0.18	0.2	0.23
Intrasystolic index	78%	83%	85%
Haemodynamic index	1.8	2	2.5
Systolic pressure, mm Hg	140	105	120
Systolic volume, ml	41	54	65
Minute volume, l	4	4.9	5.3
Mechanical action of the heart, g/cm	6,270	6,464	8,645

As an example of myocardial infarction with a severe course we present data collected during examination of patient Z. with extensive myocardial infarction who was admitted to hospital on the very first day of the disease (Table 2). A characteristic feature of this case was a progressive aggravation of cardiac failure at the end of the acute period; in spite of a certain increase in the systolic and minute volumes a considerable shortening of the expulsion phase and a sharp reduction of the haemodynamic index were noted. The patient died of a circulatory failure. The diagnosis was confirmed by autopsy. In this case, death followed a period of hyperfunction of the myocardium as a result of the latter's exhaustion.

Table 2

HAEMODYNAMIC INDICES FOR PATIENT Z.

Haemodynamic indices	1st day of disease	3rd day of disease	18th day of disease
Isometric contraction phase, sec	0.07	0.07	0.06
Tension phase, sec	0.12	0.11	0.11
Expulsion phase, sec	0.18	0.19	0.16
Intrasystolic index	72%	73%	72%
Haemodynamic index	1.5	1.9	1.4
Systolic pressure, mm Hg	95	110	140
Systolic volume, ml	30	34	42
Minute volume, l	3.6	3.9	4.9
Mechanical action of the heart, g/cm	3,272	4,295	6,114

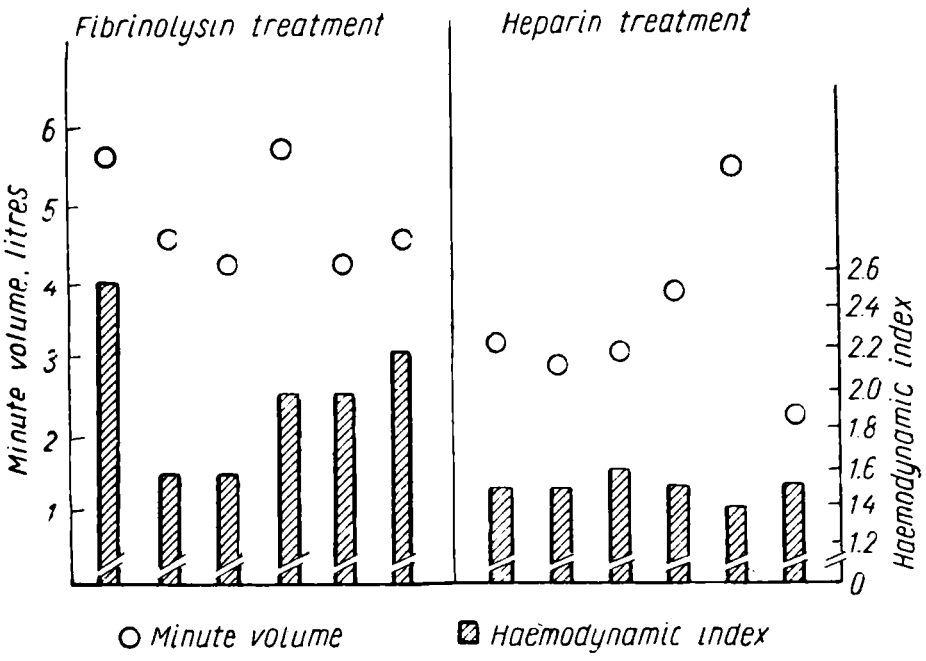

Fig. 2. Indices of the contractile function of the myocardium in patients with myocardial infarction.

One's interest is drawn to a legitimate shortening of the expulsion phase in the initial period of acute myocardial infarction, which in combination with other causes may produce a decrease in the cardiac output (Fig. 3).

In some patients, a shortening of the expulsion phase is observed for a long time, although the minute and systolic volume are growing in them. This may be accounted for by the compensatory hyperfunction of the myocardium which, as has been noted above, later leads to exhaustion of the myocardium and cardiac failure. In 4 cases, however, a shortening of the expulsion phase was not accompanied by an increase in the systolic and minute volumes. Evidently, the newly-developed myocardial hyperfunction was insufficient for maintaining the minute volume at the required level.

The clinical signs of cardiac failure were invariably revealed in the early days of the disease. Two patients were examined in the period of acute cardiac failure and showed a marked reduction of the minute volume (to 2.3 l) which was not compensated for by an increase in the peripheral resistance.

It should be noted again that disturbances in the haemodynamic indices, especially a shortening of the expulsion phase, may

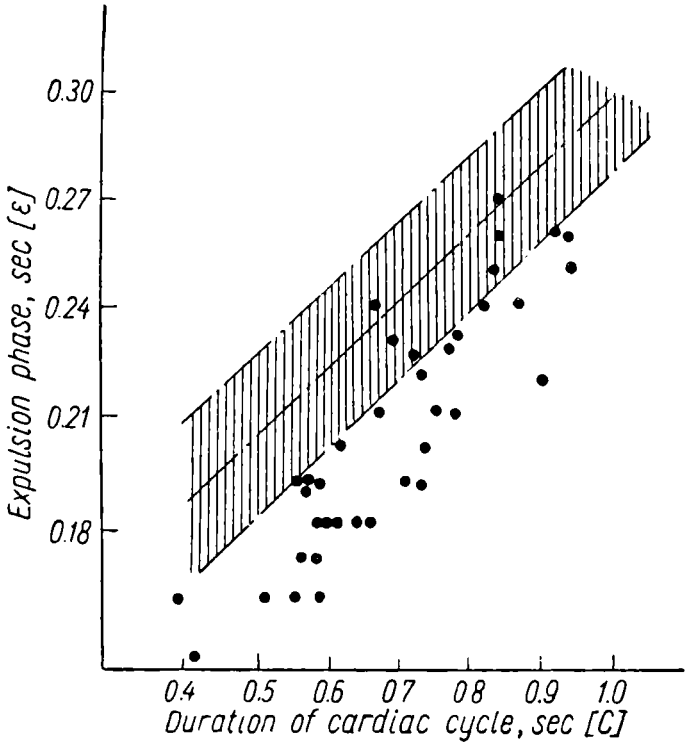

Fig. 3. Relationship between the expulsion period (E) and the duration of the cardiac cycle (C) in the initial period of acute myocardial infarction.

Shaded part of the drawing shows the limits of normal variations in the length of the expulsion period. The middle line is plotted after the formula $E=0.183C+0.112$

precede the appearance of the clinical signs of cardiac failure. For example, at the time of discharge 3 patients showed considerable changes of the haemodynamic indices without significant signs of cardiac failure. The latter, however, appeared soon after discharge of patients from the clinic in the form of attacks of cardiac asthma, liver enlargement, etc.

Without dwelling in detail on the methods of therapy we shall only point out that in addition to other remedies we used fibrinolysin which is helpful in the early hours of the disease. It is seen from Fig. 2 that in fibrinolysin treated patients the haemodynamic indices improve earlier than in those treated with heparin alone.

Thus, the following three principal mechanisms of the development of cardiac failure can be stated according to the features peculiar to the clinical course of acute myocardial infarction.

1. A sharp reduction of the minute volume not compensated by an increase in the peripheral resistance, which is observed in acute cardiac failure.

2. Inadequate hyperfunction of the myocardium evident since the early days of the disease.

3. Exhaustion of the myocardium as a result of the preceding hyperfunction.

Analysis of the duration of the systolic phases, determination of the systolic and minute volumes in account with the results of clinical and ECG investigations are important for identifying inapparent cardiac failure, timely prescription of cardiac glycosides and recommendations for rehabilitation of patients with myocardial infarction.

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EXPERIENCE WITH IN VITRO CULTIVATION OF THE AORTA OF ADULT RABBIT AND MAN

Y. E. BLOK

The principal pathomorphological processes in aortic atherosclerosis are known to take place in the intima and the adjoining media. Numerous experimental findings indicate that the primary penetration of lipids into the intima, which is the basis of the pathological process, occurs mainly from the vessel lumen with tissue lymph. The accumulation of lipids in the connective tissue of the intima is accompanied by a complex biochemical and morphological reconstruction of tissue. At the same time, in addition to the focal depositions of lipids and neutral fat there is an increase in the amount of mucopolysaccharides, lipoproteins, the formation of fibrinoid substances and, ultimately, the formation of an atheroma. An important, though inadequately known, role in this complex process is played by the cellular elements of the intima. The reproduction of fibrocytes is an indispensable reaction in the development of fibrous sclerotic plaques. In the histiocytic cells of the intima are deposited neutral fat and other lipids with the formation of isolated xanthomatous cells or their aggregations. The undifferentiated "cambial" cells of the connective tissue layer of the intima described by Langhans and studied in detail by Shchelkunov accumulate fatty substances in the form of tiny drops. The process of lipoidosis of the intima remains a mystery. "It is not clear how important is the active (biological) "swallowing" or absorption by cells of colloidal particles or how the penetration of colloidal particles into the cellular protoplasm is regulated by the physico-colloidal laws" (A. L. Myasnikov).

The peculiar structure of the intima and the adjoining layers of the media in the aorta and other larger arteries (absence of vessels and nerve endings, nutrition of cells through a very slow movement of the tissue fluid in the intercellular spaces, selective penetration of nutrients through the endothelium) makes one consider a study of the pathomorphological processes characteristic of atherosclerosis, in particular, the process of lipoidosis in tissue cultures.

The first generations of cells in tissue cultures growing on natural nutrient media (plasma and homologous blood serum) are known to retain many features in common with the maternal cells of the body. In addition to the "histiotypical" growth in a culture, i.e., preservation of the structural type of the maternal tissue (epithelial, connective, etc.) one can also observe retention in the culture of specific functional and metabolic characteristics of explanted tissue, which is the greater the more the conditions of nutrition in vitro correspond to the composition of the internal medium of the body. For example, the mammary gland tissue continues to lactate, the tissue of the endocrine glands (thyroid, pituitary), producing specific hormones.

At the same time, during in vitro study of cytological structures one should take into consideration not only their similarity but also their substantial difference from analogous structures in an intact body. This difference is not less interesting for the researcher since, as it is caused by a change in the conditions of existence of tissue elements, it reveals cell properties which were masked by the influence of the body and its integrating systems. While in an intact body one of the principal factors determining the character of cell and tissue life are the intricate relationships between different systems, above all, the nervous and humoral influences (V. G. Yeliseyev), under the in vitro conditions such factors are the physico-chemical properties of the nutrient medium surrounding the cells and the latter's hereditary, genetically conditioned properties. It is precisely the latter circumstance that accounts for the exceptional interest in the tissue culture displayed by the researcher, if the biochemical changes responsible for the pathological process are of a hereditary nature (Chang, Krooth a. Weinberg). So far as atherosclerosis is concerned, this disease is undoubtedly due to familial-hereditary predisposition (A. L. Myasnikov).

At the Institute of Therapy under the USSR Academy of Medical Sciences, experiments with in vitro cultivation of aortic tissue of adult rabbits and man have been carried out since 1962 at the suggestion of Prof. A. L. Myasnikov and Prof. I. I. Speransky. The present report describes the methods used for the cultivation of the aorta and the morphological characteristics of the cultures.

Treatment of glass vessels and mica. Carrel's vials of common or neutral glass, test-tubes, Petri dishes, 100-150 ml bottles for pouring out medium 199, vessels for keeping tissue were during one hour immersed in a hot saturated sodium bicarbonate solution, carefully washed in hot running water, rinsed in running water and put for one hour in 80 per cent sulphuric acid. Then the vessels were thoroughly (at least ten times) rinsed in running water, washed during 24 hours in running water, boiled for 20 min in distilled water, rinsed in fresh distilled water, and dried in a drying cabinet. The dried vials and test-tubes were plugged with cork stoppers, wrapped in two layers of gauze bound up and trimmed at the outer end of the stopper, and wrapped in paper. The vessels were sterilized in a drying cabinet at 180°C during $1^1/_2$ hours. The measuring pipettes, cut-off rubber nipples and stoppers were washed by an analogous method but without sulphuric acid treatment. The nipples filled with distilled water in vessels with ground-in glass stoppers and rubber stoppers wrapped in paper were sterilized in an autoclave at 2 atm pressure during $1^1/_2$ hours. The mica was washed in hot and cold running water, rinsed several times in distilled water, wiped with alcohol, placed for 24 hours in an alcohol-ether mixture and then for another 2-3 hours in another mixture of alcohol and ether. Then the mica was cut up into rectangular plates and placed in vials. The bottoms of the vials were slightly heated on a burner until evaporation of liquid.

Preparation of nutrient media. Usually the rabbit from which the aorta had been taken was also used for obtaining plasma, blood serum and, whenever required, heart extract. The air in the operating room was before the operation sterilized by means of a bactericidal lamp during one hour. The rabbit which had been starved during 24 hours was under ether anaesthesia drained of blood through the carotid artery under aseptic condition. The fur in the region of the operative field was sheared on the eve of the operation. The instruments used for the operation were sterilized by immersion in alcohol and searing in the flame of a spirit lamp. The skin was disinfected with alcohol, iodine tincture and petrol; sterile gauze squares were laid about the operative field, the tissues on the anterior surface of the neck were opened layer by layer, and the carotid artery was exposed over a length of 3-4 cm on a level with the thyroid cartilage. Dieffenbach's clamp was applied to the peripheral end of the artery which was then painted with iodine tincture; then the edge of the artery was seized with a slender pincers somewhat centrally to the clamp and the central end compressed with a wide-jawed forceps. After this an assistant severed the peripheral end of the artery and brought under it sterile tubes, which were on hand for the occasion, and filled them with blood one by one upon removal of the clamp from the central end of the artery. The tube edges before and after the collection of blood were seared in the flame of a spirit lamp, and the tubes were tightly plugged with the same stoppers. The first several drops of blood were allowed to escape. We did not use a cannula, as a rule, because its insertion into the thin carotid artery of the rabbit is difficult, makes the operation last longer and increases the danger of infecting the blood. This method usually ensures sterility during the collection of blood since at no time during the operation does the operator touch the operative field with his hands.

The blood for preparing the plasma was collected in siliconized 20-25 ml test-tubes preliminarily filled with heparin solution in medium 199 in a ratio of 5-6 U of heparin per ml of blood, whereupon the blood was centrifugated during 40 min at 2,000 rpm, the plasma was aspirated into sterile siliconized centrifugal test-tubes, which were plugged with sterile rub-

ber stoppers and kept in a refrigerator at 4°C. The small deposition of fibrin which was sometimes noted on the walls of the test-tubes with plasma did not impair the results of cultivation. The blood for preparing plasma was collected into 10 ml centrifugal test-tubes and allowed to stand at room temperature during 3-4 hours. The clot was separated from the test-tube walls, the stoppers were replaced with rubber plugs (No. 14), and the contents of the tubes were centrifugated at 2,000 rpm during 40 min.

Further, the serum was drawn into sterile centrifugal test-tubes plugged with rubber stoppers, allowed to stand at room temperature during 3 days and kept in a refrigerator at 4°C. Before use, the serum was inactivated in a thermostat at 55°C during 35 min.

Preparation of heart, spleen and chick embryo extracts. A rabbit heart resected under sterile conditions was placed in a glass containing 10 ml of medium 199; then in a box in the same glass the heart was minced by means of scissors, twice washed with medium 199, supplemented with an equal quantity of this medium, kept for 2 hours in a thermostat at 37°C (Latta, Johnson) and for 24 hours in a refrigerator at 4°C. After this, the supernatant liquid was aspirated and kept at a temperature of —20°C. Before use, the extract was centrifugated for 20 min at 2,000 rpm. We used heart extract for a period up to 3 months and noted no weakening of its stimulating properties. The rat spleen extract was prepared in an analogous manner.

For preparing the embryonal extract, the primordia of the eyes were excised from a nine-day-old chick embryo extracted under aseptic conditions, the embryo was crushed in a glass homogenizer, an equal amount of medium 199 was added, and the mass obtained was kept for one hour at room temperature, whereupon the supernatant liquid was aspirated. The extract was kept at —20°C and centrifugated before use.

Method of cultivation. Following drainage of blood from a rabbit, the ascending thoracic aorta was resected under sterile conditions and placed in a glass with a ground-in cork stopper wrapped in two layers of gauze and containing 5 ml of medium 199 with 10 per cent inactivated rabbit serum and kept until explantation (usually during $1^1/_2$-2 hours after the animal's death) in a refrigerator at 4°C. Then the adventitia was carefully cut off from the resected portion of the aorta in a box with observance of aseptic rules, the aorta was opened in Petri dishes in the longitudinal direction, washed in two portions of medium 199 containing 10 per cent of inactivated rabbit serum, cut up with a safety razor fragment jammed between the jaws of Pean's pincers into rectangular pieces about 1.5 mm in diameter, and these pieces were placed in the hollow of a thick slide containing several drops of medium 199 with 10 per cent of rabbit serum.

The rabbit plasma or a mixture of rabbit and chicken plasma (3 : 1) were diluted in a centrifugal test-tube with medium 199 in a ratio of 3 to 2; the pH of this mixture was brought to 7.0-7.2 by means of blowing the exhaled air through a Pasteur's pipette with cotton wool. Diluted plasma was spread in a thin layer over the bottom of large (dia. 5 cm) or small (dia. 3.5 cm) Carrel's vials with pre-inserted pieces of mica, so that the plasma only moistened the vial bottom. Further, two drops and one drop of extract were added to the contents of the large and the small vials respectively after which 4-6 aortic explants were placed into this still uncoagulated mixture. The vials were plugged with sterile rubber nipples and placed in a thermostat at 37°C for 24 hours. On the next day, one could observe, as a rule, partial dilution of the plasma around the pieces, therefore, the cultures were supported by an addition into each vial of 1-2 drops of plasma diluted in the above proportion and after an hour, during which period the vials had been in the thermostat, the liquid phase was added (0.4 ml into small and 0.75 ml into large vials). The vials were placed in

a thermostat at 37°C. The liquid phase consisted of 70-80 per cent of medium 199, 20-15 per cent of rabbit serum, and 10-5 per cent of extract, depending on whether a mixture of chick and rabbit plasma or rabbit plasma alone was used. Subsequently, the liquid phase was changed once in every two days. During change of the medium the pH level was regulated either by blowing exhaled air through the liquid in the vials (during alkaline change of reaction) or with the aid of a 1.4 per cent sodium bicarbonate solution in case of an acid change of reaction. When pouring out medium I99 it was supplemented with antibiotics: penicillin and streptomycin in a ratio of 100 U per ml of medium.

A total of more than 200 positive cultures of the ascending aorta of adult rabbits were produced. The term of cultivation was from 19 to 22 days. Certain cultures after their fixation in Buen's or Carnois's mixtures were stained with hematoxilin-eosin, some, after fixation in 10 per cent neutral formaldehyde, were stained by van Hison's method (with Weinhert's ferrous hematoxilin-picrofuchsin). Erlich's hematoxilin and a 0.25 per cent aqueous solution of eosin were used for staining cultures.

The best results with regard to the time of formation of the growth zone and the number of positive cultures were obtained by explantation of the aorta in a mixture of rabbit and chick plasma diluted with medium 199 as well as by use of a heart extract from an adult rabbit (see Table 1).

Twenty-four hours after explantation one could note migration of leukocytes from the depth of the pieces into the surrounding plasma. On the 2nd-3rd day there appeared initial signs of cell growth around many pieces: small branching threads extending from the periphery of the explant and consisting of 4-5 and more elongated cells forming end-to-end anastomcses, or isolated aggregations of 5-6 and more polygonal cells near the explant. By the 6th-8th day, the cultures usually reached maturity, forming a typical connective-tissue zone of growth 1.5-2 cm in diameter marked by a considerable polymorphism of its cellular elements.

After a period of 10-12 days of cultivation, at a fairly large distance from the zones of growth (2-2.5 cm) one could note the formation of foci of fibroblast-like cells which were morphologically identical with the cells of the principal zone of growth.

A similar phenomenon was observed by Ingenito and his collaborators in human aorta cultures.

During staining with hematoxilin-eosin and by van Hison's method the zone of growth appeared to consist mainly of fibroblast-like cells of variable size and shape ranging from spherical ones with a considerable cytoplasm area to narrow, markedly extended forms with elongated nuclei, at places resembling smooth muscle fibres (Figs. 1-3). Large, markedly structuralized, round or oval nuclei of these cells usually contained one or sever-

Donor of aorta	Dense phase	Liquid phase	Extract	Number explan
6 months old female rabbit	Mixture of rabbit and chicken plasma (3:1) diluted with medium 199 (3:2) and 1 drop of extract	Medium 199 70%; inactivated rabbit serum 20%; extract 10%	Chick embryo	20
Idem	Idem	Idem	Rat spleen	20
7 months old male rabbit	Idem	Idem	Heart of adult rabbit (6 months)	64
6 months old male rabbit	Rabbit plasma diluted with medium 199 (3:2) and drop of extract	Medium 199 80%; inactivated rabbit serum 15%; extract 5%	Adult rabbit heart	71

* Number of explants forming zone of growth on day of cultivation
** Percentage of positive cultures in cultivation period

al round or rod-shaped nucleoli lying in a bright field. The cytoplasm of many cells contained medium-sized vacuoli. Certain cells resembled endothelial cells in form and disposition. Sometimes, macrophage-like cells were encountered with a wide protoplasm, short thick processes and a condensed zone near the nuclei. In many cells a zone of condensed protoplasm (endoplasm) was seen around the nucleus. This zone apparently contains organoids (Fig. 3). In time (on the 8th-9th day of cultivation), the number of cells increased, and they seemed to be arranged in two zones, as it were: in the centre there was the denser, central zone, on the periphery—a looser and pale peripheral zone. It was seen under a high-power microscope that the centre of the culture was occupied by comparatively small, scintitially bound cells

Table 1

ADULT RABBIT ASCENDING AORTA CULTIVATED BY DIFFERENT METHODS

	Days of cultivation										
	1	2	3	4	5	6	7	8	9	10	Total
ber of plants*	0	0	1	1	6	2	—	—	—	—	10
cent**	0	0	5	10	40	50	—	—	—	—	50
ber of plants*	0	0	0	3	3	4	1	—	—	—	11
cent**	0	0	0	15	30	50	55	—	—	—	55
ber of plants*	0	47	9	3	3	—	—	—	—	—	62
cent**	0	73.44	87.5	92.19	96.87	—	—	—	—	—	96.87
ber of plants*	0	0	9	27	14	0	2	2	2	—	56
cent**	0	0	12.67	50.70	70.42	70.42	73.24	76.05	78.87	—	78.87

of a slightly elongated form. In the cultures a considerable number of mitoses were noted, particularly, from the 9th to 19th day. In time, the number of multinuclear and giant cells grows as does that of small round cells with dark picnotic nuclei and markedly vacuolized cytoplasm.

HUMAN AORTA CULTURE

The cultures of human aortic tissue were made of portions of the aorta resected under sterile conditions in patients undergoing operations for coarctation of the aorta performed at the Institute of Cardiovascular Surgery under the USSR Academy of Medical Sciences and at the Department of Hospital Surgery of the Sechenov First Medical Institute in Moscow. Human serum without preservants collected from several donors was supplied by the Central Institute of Haemotransfusion. Human plasma was

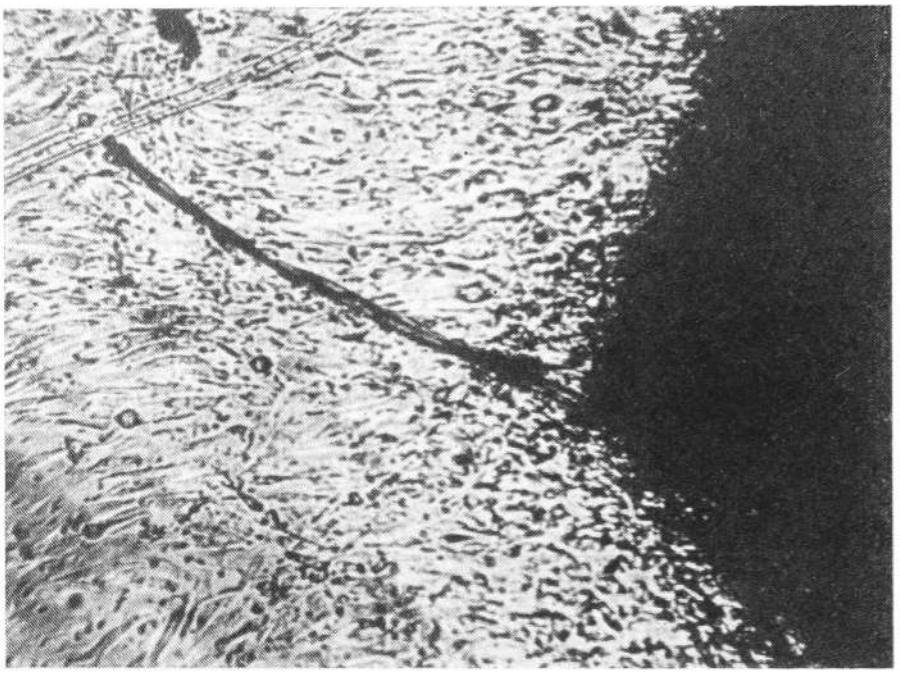

Fig. 1. Microphotograph. A culture of the rabbit aorta explanted into a mixture of rabbit and chicken plasma and chick embryo extract.
6th day of cultivation (native picture). Magnification 6.3×7

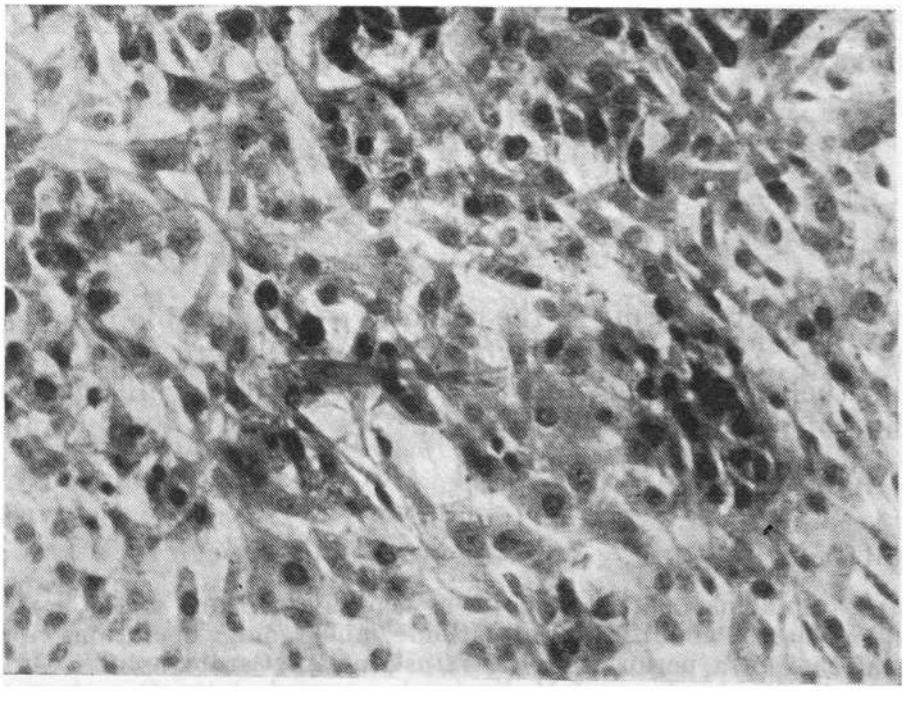

Fig. 2. Microphotograph. Same culture.
11th day of cultivation. Stained with hematoxilin-eosin. Magnification 16x7

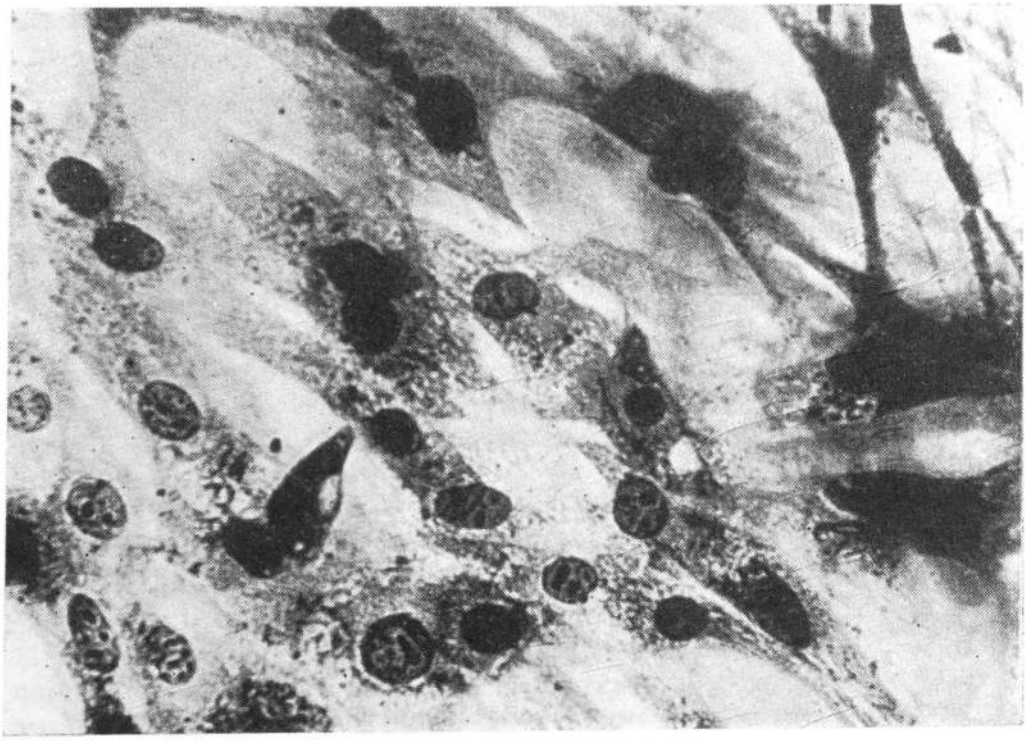

Fig. 3. Microphotograph. A culture of the rabbit aorta in homologous media. 12th day of cultivation. Stained after van Hison. Magnification 40×7

prepared from 10-15 ml of blood obtained under sterile conditions by a venous puncture from patients with hypertension in the 1st and 2nd stages. The blood was collected in siliconized centrifugal test-tubes preliminarily filled with a heparin solution in medium 199 in a ratio of 5-6 U of heparin per ml of blood. The resected portion of the aorta was placed in a vessel with 5 ml of medium 199 containing 10 per cent of inactivated human serum and was quickly delivered to the laboratory. The adventitia was cut off from the aorta, washed from blood in two portions of medium 199 with 10 per cent of human serum, cut into pieces about 1.5 mm in dia. and explanted in Carrel's large vials (four explants per vial) into a thin layer of a human and chick plasma mixture (1 : 1) diluted with medium 199 (3 : 2) and one drop of embryonic extract. Analogous results are yielded by explantation of human aortic tissue into a mixture of chicken plasma and extract to which 10 per cent of inactivated human serum is added before use. The mixture is preliminarily diluted with medium 199. The liquid phase added a day after explantation included 60 per cent of medium 199, 30 per cent of inactivated human serum, and 10 per cent of extract, and was changed three times a week. The pH of the nutrient medium was maintained at a level of 7.2-7.4. Positive cultures were also obtained from adult rabbit heart and rat spleen extracts.

Human aortic tissue was cultivated during one month at 37°C. The initial signs of cell proliferation were noted, as a rule, on the 7th day after the explantation of tissue. By the end of the cultivation period the zone of growth was formed by 25-30 per cent of explants. The cells of the zone of growth in the early stages of cultivation were loosely arranged, consisting of polygonal or round forms with 2-3 or more processes, an oval nu-

Fig. 4. Microphotograph. The aorta of patient K., age 36 years. 16th day of cultivation (native picture). Magnification 16x7

cleus containing 1-2 nucleoli and a wide finely vacuolized cytoplasm. Later, growing numbers of elongated spindle-shaped forms were noted (Fig. 4).

Until recently cultivation of the aortic tissue of adult animals and man was undertaken comparatively rarely. For example, the extensive bibliography of Murray and Kopech including all publications on tissue cultures for the period from 1884 through 1950 mentions only one study devoted to the cultivation of an adult human aorta and five studies connected with the cultivation of an adult rabbit aorta.

One of the first descriptions of the adult rabbit aorta culture is contained in a small note by Busse, 1914. The author noted the presence in the zone of growth, in addition to the spindle-shaped, stellate and smooth muscle cells, round cells after the type of mono- and polynuclear leukocytes which had undergone degenerative changes as well as fatty disintegration. Loeb and Fleisher point out in their report that pieces of aortic tissue explanted into a plasmatic clot usually do not form a zone of growth but only "survive" in the culture for several days. Haagen and Schlossberger cultivated relapsing fever spirochaetes in cultures of the rabbit aorta and other organs. The best results were

obtained in explantation of tissue into a mouse spleen and rat plasma extract mixture. In the experiments of Shibuya in Japan the best result in cultivation of the ascending aorta of an adult rabbit (up to 25 per cent of positive cultures) was produced by the use of homologous plasma and an adrenal extract. Kokubu and Pollak explanted pieces of the rabbit aorta into chicken plasma. The liquid phase included 70 per cent of Eagle's basal medium, 15 per cent of chicken embryo extract and 15 per cent of horse serum with an addition of 0.146 mg of glutamine per ml of medium. The cultures were kept for a fortnight at 38°C. The earliest and most intensive cell proliferation was observed in cultures of the aorta from rabbits with experimental atherosclerosis as well as in cultures of the ascending aorta. In the latter case, the tissue began to grow not earlier than on the 3rd-4th day after explantation.

The main difficulty in cultivation of tissue from an adult organism, especially of the dense fibrous tissue of the aorta having few cellular elements, was the need for strong influences to stimulate cell division. These influences must be capable of overcoming the inertial state which is very stable in adult tissues and of inducing the explant cells to active proliferation. In the opinion of N. G. Khlopin, the cultivation of adult tissues without using the extract is impossible in general. The embryonic extract, however, does not exert any noticeable influence on adult animal tissues.

The stimulating influence of certain adult tissue extracts, in particular the spleen extract, was described already in the early studies of Carrel. It was believed for a long time, however, that the action of the embryonic extract was stronger than that of tissue extracts. In 1939, Doljanski and his collaborators showed that the stimulating influence of certain adult tissue extracts on cell proliferation in vitro, especially extracts of smooth muscles, brain and heart tissue, is far from being inferior to that of the embryonic extract, but is even markedly stronger than the latter. A mixture of plasmas of different origin was earlier used successfully for cultivating human tissues since heparinized human plasma coagulates with difficulty and very rapidly becomes liquid in a culture (A. D. Timofeyevsky; Bucher). As is seen from the table, the most satisfactory results with regard to the time of initial cell proliferation and the number of positive cultures were obtained in our experiments with explantation of aortic tissue into a diluted mixture of chicken and rabbit plasma, as well as in the application of an adult rabbit heart extract. At the same time, in one of the experiments about 73 per cent of explants showed the initial signs of growth as early as on the 2nd day, and by the 5th day there was a zone of growth almost in

every piece, whereas during the application of a nine-day-old chicken embryo extract or a rat spleen extract the initial cell proliferation was noted in the cultures not earlier than on the 3rd-4th day, and only about 50 per cent of the cultures proved to be positive. Since cultivation in a homologous plasma is, as a rule, accompanied by a quick liquefaction of the plasmatic clot it can be presumed that addition of a heterogeneous plasma in this case also produces a stabilizing influence on the fibrin component of the clot serving as a dense substratum for the proliferating cells and thereby providing more favourable conditions for their advancement toward the periphery. Cultivation of the aorta with the use of exclusively homologous media, however, also yields quite satisfactory results (about 80 per cent of positive cultures before the 10th day of cultivation).

In cultivation of adult rabbit aorta we tried to simplify its methods as much as possible and make them suitable for application in a small, moderately equipped laboratory without specially trained ancillary personnel. The method suggested is comparatively simple and economical. All procedures involved in the preparation of nutrient media, cultivation of tissue and making histological tissue preparations can be performed by two persons (research worker and laboratory assistant) during one working day. One and the same rabbit can be used for obtaining the three principal ingredients of a nutrient medium: plasma, serum and extract. In addition, having in mind to simplify the methods, we did not use special detergents for treatment of laboratory glass and used once distilled instead of twice distilled water. For the same purpose, medium 199 instead of a saline solution was used for preparing the extract as well as for diluting the plasma and heparin.

The culture of an adult human aorta was for the first time produced by Peirce and others by cultivation of a cadaveric aorta in test-tubes so as to determine the viability of aortic transplants during prolonged storage. The aorta was explanted into a mixture of chicken plasma, homologous serum, Hanks' solution and calf embryo extract.

Aortic tissue resected during operations for coarctation of the aorta was cultivated by Ingenito and his collaborators. The pieces were explanted in vials into chicken plasma to which, after its coagulation, were added two drops of a mixture containing human serum (40 per cent), chick embryo extract (20 per cent) and Hanks' solution (40 per cent). The nutrient medium of the same composition was changed three times during a week. The initial growth in these cultures was observed during a period of 3-4 weeks and longer after explantation.

The authors give no information, however, on the number of positive cultures and the age of the donors of the aorta.

CONCLUSION

Pieces of the ascending thoracic aorta of an adult rabbit upon removal of the adventitia were cultivated in Carrel's vials at 37°C during 19-22 days. The best results were obtained by explantation of tissue into a mixture of rabbit and chicken plasma diluted with medium 199 and an adult rabbit heart extract (about 97 per cent of positive cultures with signs of initial growth on the 2nd day after explantation), as well as into a mixture of dilute rabbit plasma and heart extract (about 80 per cent of positive cultures before the 10th day).

The nutrient medium which was changed twice weekly consisted of inactivated rabbit serum (15-20 per cent), adult rabbit heart extract (5-10 per cent), and of medium 199 (80-70 per cent).

Pieces of the human aorta resected during operations for coarctation of the aorta were explanted without the adventitia into a mixture of human and chicken plasma and an extract of a nine-day-old chick embryo, as well as chicken plasma and extract to which 10 per cent of inactivated human serum had been added. The nutrient medium which was changed three times weekly consisted of human serum (30 per cent), extract (10 per cent), and medium 199 (60 per cent). Human aortic tissue was cultivated at 37°C during one month. Signs of initial growth were noted since the 7th day of cultivation. The positive cultures accounted for 25-30 per cent of the total.

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